The association between genotype and BMI, health and lifestyle indicators as well as weight loss outcomes in overweight/obese Caucasian adults.

by

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Summary

Genetic screening to improve obesity treatment outcomes is available despite the lack of conclusive evidence, specifically for Caucasian South Africans, in this regard.

The aim of this study was to investigate the association between genotype (seven polymorphisms) and body mass index (BMI), health and lifestyle indicators in a cross-sectional sample of overweight/obese Caucasian adults (n=133), as well as the association between genotype and weight loss outcomes following an intervention (n=88) using a quasi experimental study design (time-series). The intervention consisted of a 24-week conservative weight loss programme that included dietary, physical activity and behavioural components.

The primary null hypothesis for the cross-sectional sample, namely that there is no association between genotype and BMI, has not been rejected. A number of the secondary/exploratory hypotheses were rejected of which the most plausible associations (based on support by the literature and a physiological basis for the findng) are: 1) the mutant TT homozygotes of the *GNB3* C825T polymorphism may have a higher risk to develop the metabolic syndrome (MetS) as they had significantly higher fasting triglyceride and glucose levels, a higher number of traits that met the diagnostic cut-off criteria for MetS and higher number of these subjects was diagnosed with MetS compared to the wild-type C-allele carriers; and 2) subjects with mutant alleles of either the *FTO* rs1421085 or rs17817449 polymorphisms may have poorer eating behaviours (a higher rigid control, habitual and emotional disinhibition, perceived hunger and internal locus for hunger) and higher intake of high-fat foods.

The primary null hypothesis for the intervention sample, namely that there is no association between genotype and weight loss outcome, was not rejected for the *FABP2* Ala54Thr, *INSIG2* rs7566605, *FTO* rs1421085, *ADRB3* Trp64Arg and *GNB3* C825T polymorphisms. However, it was rejected in some instances indicating the following associations: 1) The wild-type TT homozygotes of the *FTO* rs17817449 polymorphism lost significantly more weight during the first two months of the program compared to the mutant allele carriers (this is a novel finding); 2) The wild-type Arg16Arg homozygotes of the *ADRB2* Arg16Gly polymorphism lost significantly more weight during the first month of the program compared to the mutant allele carriers (this finding is supported by one other intervention study); 3) Subjects with a mutant C-allele of the *INSIG2* rs7566605 polymorphism and a mutant Gly16-allele of the *ADRB2* Arg16Gly polymorphism lost significantly less weight over the six month intervention period (this is a novel gene-gene interaction finding). A number of secondary/exploratory hypotheses were rejected, of which the most plausible finding include that the improvement in emotional disinhibition in the wild-type TT subjects

of the *FTO* rs1421085 polymorphism was associated with a more pronounced decrease in BMI over the six month weight loss period.

The integration of the results from this study with the literature indicates that there is insufficient evidence at this stage for genetic screening of the polymorphisms investigated in this study and the provision of evidence-based personalized recommendations for weight loss in obese individuals. It is recommended that these associations should be viewed as priority in future research.

Opsomming

Genetiese sifting om die resultate van vetsug behandeling te verbeter is beskikbaar ten spyte van ‘n tekort aan genoegsame bewyse, spesifiek ten opsigte van Kaukasiërs van Suid-Afrika.

Die doel van hierdie studie was om die assosiasie tussen genotipe (sewe polimorfismes) en liggaamsmassa indeks (LMI), gesondheid en lewenstyl indikatore in ‘n dwarssnit (cross-sectional) steekproef van oorgewig/vetsugtige Kaukasiër volwassenes (n=133) te ondersoek, asook die assosiasie tussen genotipe en gewigsverlies uitkomste na afloop van ‘n intervensie (n=88) in ‘n kwasi-eksperimentele studie ontwerp (tyd-reeks). Die intervensie het bestaan uit ‘n 24-week konserwatiewe gewigsverlies program met dieet, fisieke aktiwiteit en gedragskomponente.

Die primêre nul hipotese vir die dwarsnit steekproef, naamlik dat daar geen assosiasie tussen genotipe en LMI is nie, is nie verwerp nie. ‘n Aantal sekondêre/spekulatiewe hipotesis is verwerp waarvan die mees geloofwaardige assosiasies (gebasseer op ondersteuning van die literatuur en ‘n fisiologiese basis vir die bevinding) die volgende insluit: 1) die mutante TT homosigote van die *GNB3* C825T polimorfisme het moontlik ‘n hoër risiko vir die ontwikkeling van die metaboliese sindroom (MetS) aangesien hulle betekenisvolle hoër vastende trigliseriede en glukose vlakke gehad het, ‘n grooter aantal kenmerke gehad het wat aan die diagnostiese afsnykriteria vir MetS voldoen en ‘n grooter aantal van hierdie persone was met MetS gediagnoseer in vergelyking met die wilde-tipe C-alleel draers; en 2) persone met die mutante allele van die *FTO* rs1421085 of rs17817449 polimorfismes het moontlik ‘n swakker eetgedrag (‘n hoër rigiede kontrole, gewoonte en emosionele disinhibisie, waarneembare honger en interne lokus van honger) en ‘n hoër inname van hoë-vet voedsel.

Die primêre nul hipotese vir die intervensie steekproef, naamlik dat daar geen assosiasie tussen genotipe en gewigsverlies uitkomste is nie, is nie vir die *FABP2* Ala54Thr, *INSIG2* rs7566605, *FTO* rs1421085, *ADRB3* Trp64Arg en *GNB3* C825T polimorfismes verwerp nie. Dit was egter in sommige gevalle vir die volgende assosiasies verwerp: 1) Die wilde-tipe TT homosigote van die *FTO* rs17817449 polimorfisme het betekenisvol meer gewig in die eerste twee maande van die program verloor in vergelyking met die mutante alleel draers (dit is ‘n nuwe bevinding); 2) Die wilde-tipe Arg16Arg homosigote van die *ADRB2* Arg16Gly polimorfisme het betekenisvol meer gewig gedurende die eerste maand van die program verloor in vergelyking met die mutante alleel draers (hierdie bevinding word ondersteun deur een ander intervensie studie); 3) Persone met ‘n mutante C-alleel van die *INSIG2* rs7566605 polimorfisme en ‘n mutante Gly16-allele van die *ADRB2* Arg16Gly polimorfisme het minder gewig tydens die ses maande intervensie periode verloor (dit is ‘n nuwe geen-geen interaksie bevinding). ‘n Aantal sekondêre/ spekulatiewe hipoteses is verwerp, waarvan die mees geloofwaardigste bevinding insluit dat ‘n verbetering

in emosionele disinhibisie van die wild-tipe TT persone van die *FTO* rs1421085 polimorfisme geassosieer was met ‘n meer prominente daling in LMI oor die ses maande gewigsverlies periode.

Die integrasie van die resultate van hierdie navorsing met die literatuur dui aan dat daar op hierdie stadium onvoldoende bewyse vir genetiese sifting en die voorsiening van bewys-gebasseerde persoonlike aanbevelings vir gewigsverlies in vetsugtig individue bestaan vir die polimorfismes wat ondersoek is. Dit word aanbeveel dat hierdie assosiasies as prioriteit in toekomstige navorsing beskou moet word.

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Chapter 1

Introduction

1.1 Problem identification and motivation for study

In 2003, the World Health Organization (WHO) estimated that globally more than 1 billion adults were overweight with at least 300 million being obese (WHO 2003). Obesity has reached epidemic proportions in many countries and is still escalating at an alarming rate world-wide, affecting children and adults in both developed and developing countries, including South Africa (WHO 2003, WHO 2010, DoH 2007).

According to the second South African Demographic and Health Survey (SADHS), obesity prevalence (BMI $\geq$ 30 kg/m^2) in adult South Africans older than 15 years is 8.8% for men and 27.4% for women, while a further 21.0% men and 27.5% women are overweight (BMI$\geq$25 and <30 kg/m^2) (DoH 2007). For women obesity is a problem across all races with the prevalence being 24.8% for Indian, 13.7% for Caucasian, 26.5% for Coloured and 28.5% for Black females. The obesity prevalence for Caucasian males (23.0%) is higher than the prevalence for Indian (10.9%), Coloured (14.9%) and Black (7.1%) men (DoH 2007). A comparison of the weight status results from the first (conducted in 1998) to the second SADHS (conducted in 2003) revealed that this situation has remained unchanged over the five year period (DoH 2007).

The high overweight and obesity prevalence is concerning as it is known that obesity is associated with increased morbidity and mortality risk (Brown *et al*. 2009). Obesity is an intermediate risk factor for the development of chronic diseases of lifestyle (CDLs) including cardiovascular disease, stroke, cancer and Type 2 diabetes (WHO 2005, WHO 2008, Brown *et al*. 2009). These chronic and life-threatening diseases are often accompanied by a range of other non-fatal but debilitating conditions such as infertility, dermatological problems, respiratory difficulties, osteoarthritis and gout that affect the immediate quality of life (Brown *et al*. 2009). Furthermore, obese individuals often have to contend with factors such as social stigmatization and discrimination, especially in Western societies, that contribute to psychological problems (Simon *et al*. 2006). These include the development and progression of a low self-esteem, body image problems, binge eating (Stunkard & Sobal 1995), loss of self-confidence, depression and lower levels of happiness (summarized by Franz 1998).

Because of the negative consequences associated with obesity, research has extensively focussed on the development and implementation of various obesity prevention and treatment/management strategies, which is still ongoing (Aronne *et al*. 2009, Wolf & Woodworth 2009). However, despite these efforts to address the problem and the fact that it has been shown that small weight losses of five to ten percent of initial weight are associated with clear health benefits (Seagle *et al.* 2009), the prevalence of obesity remains high in many countries and is still increasing worldwide (WHO 2010). This situation can be ascribed to the fact that prevention and treatment/management of obesity have proven not to be simple tasks most probably due to the heterogeneous nature of the disease that has yet to be fully elucidated

(Rosmond 2004, WHO 2000, Dahlman & Arner 2007). Although it is known that obesity results when energy intake chronically exceeds energy expenditure, creating a positive energy balance (Papas *et al*. 2007), the pathophysiology of the condition is complex (Romao & Roth 2008) and the causes of the positive energy balance are multifactorial. It is now acknowledged that effective obesity prevention and management should address the root causes of the condition that can include any combination of genetic, metabolic, biochemical, psychological, physiological and environmental factors (Rosmond 2004). In terms of environmental factors, the concept 'obesogenic' environment has emerged to describe aspects of the built and social environments that promote the adoption of 'obesogenic' behaviour that includes the consumption of large portion size meals, unhealthy high-fat and refined carbohydrate diets and physical inactivity (Bouchard 2007). However, it is well known that not all individuals living in an obesogenic environment and engaging in obesogenic behaviour will gain weight (Loos & Rankinen 2005, Chung & Leibel 2008). Furthermore, it has also been observed that obese individuals following the same obesity treatment strategies do not necessarily react the same to this treatment (Kaput & Rodriguez 2004, DeBusk *et al*. 2005, Hainer *et al*. 2008, Reilly & DeBusk 2008). Studies on monozygotic and dizygotic twin pairs have confirmed that exposure to the same controlled environment has led to the same weight loss in response to a weight reduction programme in twins carrying identical genes (monozygotic twins). However, differences in weight loss between twin pairs or in dizygotic twins are clearly evident (Bouchard *et al*. 1994, Hainer *et al*. 2000). Although the fact that heritability does contribute anything from 5% to 90% to the variation in human adiposity (Loos & Bouchard 2003, Romao & Roth 2008) has long been recognized, the advent of nutritional genomics in the early 2000s provided a new perspective on this relationship (Loktionov 2003, Kaput & Rodriguez 2004). The nutritional genomics perspective proposes that when individuals engage in the same obesogenic behaviour, those who have genetic mutations that promote the development of obesity will gain more weight and probably develop obesity, while those without the genetic predisposition for obesity will gain little if any weight (Loos & Bouchard 2003, Loos & Rankinen 2005, Chung & Leibel 2008).

The challenge posed by the nutritional genomics perspective lies in the fact that any number and combination of susceptibility genes may be interacting in numerous ways with any combination of environmental and other health factors to result in the obese phenotype (Chung & Leibel 2008, Marti *et al*. 2008, Romao & Roth 2008). To date a large number of candidate genes and genetic markers that are associated with a variety of obesity-related phenotypes have been identified (Rankinen *et al*. 2006). According to the latest obesity gene map, the number of human obesity quantitative trait loci (QTLs) for obesity-related phenotypes is 253 generated from 61 genome-wide scans. A total of 52 of these genomic regions are supported by two or more studies. From candidate gene studies, 426 positive associations with obesity related phenotypes have been reported for 127 candidate genes. Of these candidate genes, 22 have shown associations with obesity-related phenotypes in at least five studies (reviewed by Rankinen *et al*. 2006).

It has been proposed that the science of nutritional genomics could result in gene based susceptibility screening for a specific phenotype, also referred to as predictive genetic testing (PGT). PGT refers to the screening of healthy individuals in the general population to identify those who are at risk of developing a multifactorial chronic disease such as obesity, cancer, heart disease etc. based on the presence of mutant polymorphisms in several genes (Evans *et al*. 2001). Furthermore, genetic screening could also be used to predict individual response to treatment in those with already developed risk factors or diseases. The outcomes of genetic screening are subsequently applied to personalize nutrition (Joost *et al*. 2007), involving genotype tailored dietary recommendations for the individual in order to prevent, treat or even cure a disease (Boehl 2007). Proposed benefits of genotype-based personalized nutritional advice for chronic conditions such as obesity include the following: Firstly, genotyping for early identification of at risk individuals without manifested risk factors, may result in early introduction of targeted prevention when compared to current practices of identification and consequent treatment of manifested risk factors for diseases (DeBusk *et al.* 2005, Joost *et al*. 2007, Stover & Caudill 2008). Joost *et al*. (2007) maintain that such preventative advice may be especially beneficial for conditions that are known to have a long latency period in the development of associated complications. Secondly, Joost *et al*. (2007) argue that genotype based disease prevention/treatment may result in more cost-effective treatment as successful interventions can be targeted at those who are most likely to benefit from them. Thirdly, genotype results may motivate at risk individuals to change their lifestyle to comply with necessary dietary advice (Joost *et al*. 2007). It is thus proposed that genetic screening will enable dietitians and other health professionals to ultimately move away from generalized nutrition recommendations to prescribing more genotype-based personalized diets and other lifestyle recommendations to prevent/treat disease (DeBusk *et al*. 2005, Trujillo *et al*. 2006).

Although genetic screening has been used effectively in the past to screen for relatively uncommon monogenic disorders, it has become increasingly available on the market for chronic disease prevention/management. In many countries, including South Africa, companies have started marketing genetic screening, through health care providers or directly to the public, even in the form of home-testing kits (DeBusk *et al*. 2005, Jakobsdottir *et al*. 2009, www.dnadiet.co.za). The tests are often accompanied by customized recommendations for the intake of specific vitamins and foods according to each individual's genotype (www.quackwatch.org). There is no doubt that economic forces played a role in creating a strong drive for the increased availability of genetic screening (Baird 2001). Baird (2001) comments that in countries where there is sufficient wealth to provide a potential market of consumers, inappropriate overuse could become a challenge.

In light of these developments it is important to emphasize that as is the case with all clinical practice in dietetics, genetic screening for personalization of dietary recommendations for obesity

prevention/treatment needs to be evidenced-based (Gray & Gray 2002). In 2002 Nieters *et al*. (2002) mentioned that research regarding the link between obesity related genes, phenotype markers and environmental factors (e.g. dietary intake and physical activity) and how this would translate into formulation of effective obesity prevention and management strategies at lifestyle level, was still in its infancy. At the time it was also suggested by a number of researchers that these questions should become the focus of nutritional genomics research worldwide (Patterson 1999, Kaput & Rodriguez 2004). Loktionov (2003) mentioned that these questions should be investigated in humans using association or linkage studies with obesity phenotypes or even better, in actual intervention studies to assess the effect of genotype on the response to dietary and possibly other life-style changes, bearing in mind the genetic heterogeneity amongst different race groups. Although the effect of some candidate genes on weight loss outcomes have been investigated since these suggestions were made (reviewed by Hainer *et al*. 2008), the latter authors concluded that the evidence is not yet sufficient to support evidence-based practice in this field and recommend that more research is necessary before effective personalized obesity prevention or treatment (weight loss diets) strategies for obese individuals can be suggested. More specifically, although associations for 127 candidate genes with one or more obesity phenotype indicator have been reported, the effect of only very few of these genes on weight loss outcomes have been investigated in clinical trials (Hainer *et al*. 2008). None of the trails that have been completed were conducted on South Africans.

For the contention of Simopoulos (2010) that "every physician and other relevant health care professionals will need to utilize genetic information to recommend diet, physical activity and pharmacotherapy" to become reality as part of evidence-based practice for obesity prevention/treatment in South Africa, research among South African population groups in this regard is imperative. Such research will also contribute to informing health care professionals and the public regarding the appropriateness of currently available genetic screening for obesity prevention/treatment.

1.2 Aims and objectives

Aim:

To investigate the association between genotype and BMI, health and lifestyle indicators as well as weight loss outcomes in overweight/obese Caucasian[1] adults[2].

[1] *A parallel group of South African Zulu overweight/obese adults is currently under investigation*
[2] *These genotype associations were investigated in a cross-sectional and an intervention sample (see Section 3.1 for detail).*

Objectives:

Cross-sectional sample of overweight/obese Caucasian South African adults:

1. To determine and describe the physical health of subjects in terms of the prevalence of metabolic syndrome (MetS) and each MetS trait.
2. To describe the association between BMI and health (perceived weight history, MetS prevalence, MetS traits and psychological health) and lifestyle (physical activity, dietary intake and eating behaviour) indicators.
3. To investigate the association between genotype and BMI, health and lifestyle indicators:
   - Determine the genotype and allele frequencies of the following obesity-related polymorphisms: *FABP2* Ala54Thr, *INSIG2* rs7566605, *FTO* rs1421085, *FTO* rs17817449, *ADRB3* Trp64Arg, *ADRB2* Arg16Gly and *GNB3* C825T.
   - Determine the association between genotype and BMI, health and lifestyle indicators.
   - Determine the effect of the interaction between genotype and health and lifestyle indicators on BMI.

Intervention sample of overweight/obese Caucasian South African adults:

4. To assess the effectiveness of a conservative weight loss programme in terms of
   - Weight and BMI reduction over the total 24 week intervention period.
   - Changes in selected health and lifestyle indicators that were the focus of strategies included in the weight loss programme from baseline to 16 week follow-up.
5. To investigate the association between genotype and conservative weight loss outcomes:
   - Determine the association between genotype and treatment outcome (change in weight and BMI) at four, eight, 12, 16 and 24 weeks of the intervention period.
   - Determine the effect of the interaction between genotype and change in process outcomes (selected health and lifestyle indicators that were the focus of strategies included in the weight loss programme) on change in BMI over the 24 week intervention period.

6. To integrate the genotype associations found in objectives 3, 5 and existing evidence to elucidate the potential role of genetic screening for polymorphisms included in this study in the formulation of personalized obesity treatment strategies for overweight/obese Caucasians.

1.3 Definition of terms

For the purposes of this research health and lifestyle indicators reflect the following (see Chapter 3 for details):

Health indicators:

- Physical health: MetS traits (waist circumference, blood pressure, glucose, triglyceride and HDL levels.) and the diagnoses of MetS.
- Weight history: perception of weight during childhood, adolescence and young adulthood.
- Psychological health: general psychological well-being, depression and self-esteem.

Lifestyle indicators:

- Physical activity: Work, sport and leisure-time physical activity
- Dietary intake: Frequency of intake of energy-dense indicator food groups namely high fat foods, take-away foods, energy-dense snacks and energy-dense drinks.
- Eating behaviour: Dietary restraint, disinhibition and perceived hunger.

Conservative treatment refers to a six month weight loss programme consisting of dietary, physical activity and behavioural strategies (see p132 for details)

Weight loss outcomes refer to:

- Treatment outcomes: Changes in weight and BMI over a 24 week intervention period.
- Process outcomes: Changes in selected health (psychological health) and lifestyle (physical activity and eating behaviour) indicators that were the focus of strategies included in the weight loss programme

1.4 Outline of thesis

In Chapter 2 a review of the literature is presented that firstly focuses on a brief overview of the etiology of obesity followed by an in depth discussion of each gene and polymorphism investigated in this study (literature up until January 2010 were included). This is followed by a detailed description of the methods and experimental procedures used in this study in Chapter 3. The results, discussion and conclusions of the cross-sectional sample to meet objectives 1 to 3 of this study are presented in Chapter 4. The results, discussion and conclusions of the intervention sample to meet objectives 4 and 5 follow in Chapter 5. Finally, an integrative discussion of Chapters 4 and 5 to meet objective 6 is presented in Chapter 6. The references for all the above-mentioned Chapters appear in Chapter 7.

Chapter 2

Literature review

The etiology of obesity, including the genetics of obesity, are the core concepts discussed in the first section of this literature review. The focus in the first section is on the role of dietary intake, physical activity, the built-environment, socio-economic status, education, culture, behavioural, psychological and biological factors in obesity development. Following this, the genetics of obesity, including monogenic and polygenic forms thereof are described. The discussion on each gene included in this research, provides specific reference to the characteristics of the gene, the protein expressed by the gene and the physiological function thereof, the mutation(s) selected for investigation in this research as well as the functionality of the mutation and the reported genotype and allele frequencies. This is followed by tabulated summaries and discussions on the association of each selected mutation with weight status, fat distribution, glucose homeostasis, plasma lipid profile, blood pressure and outcome of weight loss intervention programmes.

2.1 Etiology of obesity

Obesity results when energy intake chronically exceeds energy expenditure, creating a positive energy balance (Papas *et al*. 2007). However, the pathophysiology of obesity is complex (Romao & Roth 2008) and the causes of the positive energy balance are multifactorial and not fully understood, with several lifestyle factors manifesting together in a genetically susceptible individual to produce weight gain and obesity development (Rosmond 2004, WHO 2000, Dahlman & Arner 2007). In almost all cases obesity development is characterized by an interaction between genetics and environmental factors over time (Romao & Roth 2008).

The extra energy from a positive energy balance is stored as fat in adipose tissue and results in gradual weight gain over years and consequent development of obesity (Dahlman & Arner 2007). According to the WHO (2000) gaining weight can be divided into the following three phases:

- A **pre-obese static** phase during which long-term energy balance is experienced and weight remains constant.
- A **dynamic** phase during which weight gain occurs as a result of a prolonged positive energy balance. This phase may continue for several years and is often recognized by considerable fluctuations in weight (weight-cycling) as the individual tries to lose weight. During this phase an energy imbalance of as little as 80 kJ in excess per day can cause a 0.89 kg body weight gain in an average person per year or 4.4 kg in five years (Cohen 2008). The rate of weight gain can eventually decrease as a result of physiological adaptations such as changes in basal metabolic rate (WHO 2000).
- An **obese static** phase during which energy balance is regained, however this energy balance now occurs at a higher weight than the weight held during the pre-obese static phase, as a result of the weight gain that occurred in the dynamic phase (WHO 2000). In the obese static phase the body appears to defend this new (higher) body weight referred to as the new "settling point" (Peters 2006). During adult life several settling points are defended by the body as individuals go through dynamic weight gain phases followed by a weight stable phase (WHO 2000).

According to Bouchard (2007) the potential contributors to the higher obesity prevalence seen over the past decades can be grouped under four major headings, namely the built environment, social environment, behaviour and biology. Built environment factors contribute to obesity development when it causes physical inactivity and high energy consumption e.g. increased use of automobiles, building design favouring increased access to fast-food outlets and lack of safe sidewalks. The social environmental factors that increase the risk for obesity development include marketing, advertising and cultural influences. Together the built and social environments result in the phenomenon that is referred to as the 'obesogenic' environment in both developed and in most developing areas of the world. This obesogenic environment promotes the adoption of obesogenic behaviour that includes the consumption of large portion size meals, high-fat diets, high sugar intake, many hours spent watching television, playing video games or sitting at a computer. Consequently, in a population exposed to this obesogenic environment and that partakes in the same obesogenic behaviour, those with a higher biological predisposition to develop obesity (with genetic polymorphisms favouring weight gain) will gain more weight. However, in comparison to them, others with an intermediate, very low or non-existent predisposition will gain less or no weight respectively (Bouchard 2007).

2.1.1 Dietary intake

Experimental studies in animals and humans have repeatedly shown that dietary factors, especially fat and energy intake, are associated with obesity development (WHO 2000). The term, obesogenic foods, has been used to collectively refer to those components in the diet that might cause obesity. However, at this point in time no standard definition for obesogenic foods is available. Dietary components referred to as obesogenic by various authors, include the following: a higher intake of energy-dense food (Black & Macinko 2008), fats (Bray *et al*. 2004a, Rosmond *et al*. 2004), refined carbohydrates (Gross *et al*. 2004), high-fructose corn syrup (Bray *et al*. 2004b), sweetened beverages (Malik *et al*. 2006, Gibson 2008, Wolff & Dansinger 2008) and a low carbohydrate diet (<47% of total energy) (Merchant *et al*. 2009). Furthermore, eating fast foods (Black & Macinko 2008), eating away from home (McCrory *et al*. 1999, Popkin 2006), eating larger portions sizes (Nielsen & Popkin 2003, Popkin 2006) and an inappropriate meal pattern and snacking (Popkin 2006, Marín-Guerrero *et al*. 2008, Howell *et al*. 2009) may be referred to as obesogenic eating behaviours. A discussion of these dietary components and the association thereof with the development of obesity is presented in the following sections.

Macronutrient content of diet

It is well known that the extent to which excess energy is stored depends on the macronutrient content of the diet as the metabolic and physiologic characteristics and effects of the different macronutrients differ (Table 2.1). When excess energy is consumed, priority for oxidization goes to those macronutrients with no or limited storage capacity within the body. Alcohol is always oxidized first because it is toxic to the body and the body has no storage capacity for it. Second in line for oxidation is protein, as the body has a limited storage capacity and because amino acid balance is tightly regulated so that any excess is oxidized. Carbohydrate is third in line, as the body can store only limited amounts of carbohydrates in the form of glycogen in the liver and muscle tissue

(WHO 2000, Peters 2006). Liver glycogen can be released to keep blood glucose levels constant for instance during overnight fasting, while muscle glycogen supplies glucose to working muscles (Peters 2006). In humans, excess carbohydrates are not usually stored as fat. However, this can occur when the diet supplies very little fat and excessive amounts of carbohydrates over time. Last in line is fat, as the body has virtually an unlimited storage capacity for fats in the adipose tissue, while fat intake does not markedly increase fat oxidation, thus favouring adipogenesis. It is also important to note that the storage of fat requires very little energy and the storage process is thus highly energy efficient. Thus, when considering the metabolism of the macronutrients, it is evident that dietary fat intake favours fat storage when a positive energy balance exists for the mentioned reasons (WHO 2000, Peters 2006).

Table 2.1: Metabolic and physiologic characteristics and effects of macronutrients

	Protein	**Carbohydrates**	**Fat**
Ability to bring eating to an end	High	Intermediate	Low
Ability to suppress hunger	High	High	Low
Contribution to daily energy intake	Low	High	High
Energy density	Low	Low	High
Storage capacity in the body	Low	Low	High
Metabolic pathway to transfer excess intake to another compartment	Yes	Yes	No
Autoregulation (ability to stimulate own oxidation on intake)	Excellent	Excellent	Poor

From WHO (2000).

Fat intake

A higher fat intake has previously been associated with a higher BMI in Black South Africans (Kruger *et al*. 2002, Vorster *et al*. 2000). Possible explanations for the link between obesity development and a high-fat intake may include the fact that dietary fat provides more energy per gram when compared to the other macronutrients (Peters 2006). Consequently, passive overconsumption can easily occur when individuals are exposed to high-fat foods. Furthermore, the high palatability that fat provides to a meal has a stimulatory effect on food intake. In addition it is thought that the signals controlling appetite and food intake may be too weak or too delayed in response to a fatty meal to control food intake. Consistent high intake of fatty foods may overwhelm these signals and influence long-term regulatory processes stimulating an inability to respond to overeating (WHO 2000).

Palatability

Sweetness and fat promote the palatability of foods, which is associated with increases in the rate of eating and the sense of hunger during meals (WHO 2000). Fat provides a pleasurable mouth feel, while sweetness provides the easy and most powerful recognisable enjoyable tastes. It has been shown that obese women prefer fat-sweet mixtures which may be a contributing factor to weight gain (WHO 2000). In a review article by Mattes

(2008) it was indicated that the consumption of palatable foods is associated with a greater energy intake and weight gain.

Carbohydrate intake

It has been shown that diets providing between 290 to 310g of carbohydrates per day (between 47 to 64% of total energy per day) are associated with the lowest risk for overweight or obesity (Merchant *et al*. 2009). However, a low carbohydrate diet consisting of less than 47% of total energy intake seems to be associated with obesity development (Merchant *et al*. 2009). Such a low carbohydrate diet would normally provide more fat compared to the higher carbohydrate diet to make up the energy composition. As summarized in Table 2.1, carbohydrates, especially complex and low GI carbohydrates have the ability to suppress hunger and have a lower energy density and storage capacity in the body compared to fat (WHO 2000).

High fructose corn syrup

It has been argued that the increased production and intake of high fructose corn syrup (HFCS) may be associated with the increasing prevalence of obesity (Bray 2008, Duffey & Popkin 2008). Since the 1970s, lower farming prices have led to a decrease in the price of maize, making it an inexpensive starch that could be converted in to a highly sweet and very cheap liquid known as HFCS, using the new isomerase technology. HFCS is now commonly used in the manufacturing of soft drinks and other highly processed foods. Therefore, since the early 1970s a steep rise in HFCS was noticed in the food supply chain (Bray 2008). It is evident that HFCS has rapidly replaced sugar in manufactured products such as sports drinks, high-fat milk, salad dressings, sweetened coffee and tea, hamburgers, breads, cereals and desserts and almost entirely replaced the sugar in fruit juices and soft drinks (Bray 2008, Duffey & Popkin 2008). From a review of 12 studies by Bray (2008) it was evident that 10 indicated a positive association between HFCS containing soft drinks and energy intake. The author concluded that soft drink consumption usually adds additional kilojoules to the daily energy consumption, as individuals normally do not lower the intake of another food to compensate for the energy content of the soft drink (Bray 2008). Furthermore, data from the United States of America indicates that less water and milk are consumed at this point in time, while regular soft drinks and beer are the first and second most frequently consumed beverages (Nielsen & Popkin 2004, Popkin 2006, Wolf *et al*. 2008).

Fast-foods

The consumption of fast-foods may also be associated with greater weight gain and obesity development (Duffey *et al*. 2007, Larson *et al*. 2009). Although it can be argued that fast-foods were available 50 years ago when obesity prevalence was very low, today many individuals do not consume fast-foods as an occasional meal or treat but rather include it is as a regular addition to their diet. It is known that the accessibility and consumption of fast-foods has grown significantly over the past two decades with famous outlets such as McDonalds and Kentucky Fried Chicken spreading rapidly across the globe (Popkin 2006). Meals from these outlets are often irresistible for a large section of the population, as they are intensely marketed, highly palatable and available at relatively cheap prices compared to other ready meals (WHO 2000). In general, fast-

foods are low in complex carbohydrates and micronutrients, high in fat, energy-dense, high in glycemic load, excessive in portion sizes and not entirely satisfying (WHO 2000, Rosenheck 2008). Days when individuals eat at fast-food restaurants are characterized by a higher intake of soft drinks and chips and a lower intake of vegetables, cereals and milk compared to days when they do not eat at these restaurants (Paeratakul *et al*. 2003). Fast-food consumption has been associated with higher energy intakes, BMIs, greater long-term increases in weight and a higher risk of being obese and having a poor diet quality (Bowman & Vinyard 2004, Schröder *et al*. 2007, Stender *et al*. 2007, Black & Macinko 2008, Rosenheck 2008). In a 15 year follow-up study it was shown that individuals who consumed fast-foods more than twice a week gained more weight over 15 years than those who eat fast-foods less than once a week (Pereira *et al*. 2005).

Portion size

An increase in portion sizes of food and beverages consumed is another food environmental change that has occurred over the past 30 years (Cohen 2008). It has been reported that the typical restaurant now serves portions that are two to five times bigger than is required for an individual to remain in energy balance (Nestle 2003, Nielsen & Popkin 2003, Young & Nestle 2007). Factors that contribute to this phenomenon from food consumed at individual's homes, include that more food is available from the larger packaging size used together with the availability of larger supermarkets or warehouse stores stocking larger quantities of food (WHO 2000). It has been shown that obese individuals eat larger portion sizes when compared to lean individuals (Berg *et al*. 2009) and growing evidence suggest that people will eat more when presented with larger portion sizes (Wardle 2007).

In Western societies the accepted norm of eating large portion sizes contributes to active overeating, thus a high energy intake due to eating a large amount of food. Normally food becomes less pleasant as more is consumed, which is referred to as sensory-specific satiety. However, this principle can be overridden when a shift to another food choice during the meal occurs. For instance, at buffet meals overconsumption can often occur when a variety of foods are available. Furthermore, the fact that individuals perceive that they get better value for their money when buying upsize meals or partake in all-you-can-eat buffets from restaurants and fast-food outlets, contributes to the intake of larger portion sizes (Gee *et al*. 2008).

Passive excessive intake refers to the consumption of energy-dense meals. Fisher *et al*. (2007) have shown that when individuals are presented with different starters or meals consisting of the same amount of food but with varying energy densities, a higher energy intake results from eating the energy-dense starters or meals. This can be explained by the fact that individuals do not adjust the amount of food taken in according to the energy density of a meal (Fisher *et al*. 2007). The fact that energy-dense foods is known to be more palatable and less satiating compared to low energy-dense foods (Drewnowski *et al*. 2004) contributes to excessive intake. Therefore the portion size of the food or meals eaten during the day may be small or similar to a normal size but a higher energy intake and consequent weight gain occur due to the energy density of the food (Gee *et al*. 2008).

Eating pattern

The regularity of a person's eating pattern may be associated with obesity development (Timlin & Pereira 2007, Howell *et al*. 2009). It has consistently been shown that skipping *breakfast* is associated with weight gain and a higher risk of being obese (Timlin & Pereira 2007, Marín-Guerrero *et al*. 2008, Berg *et al*. 2009). Individuals who eat a high fibre breakfast regularly have a better overall diet quality and micronutrient intake, a lower fat intake, less uncontrollable nibbling episodes during the day and less incidence of between meal hypoglycemia (Nicklas *et al*. 2001, Timlin & Pereira 2007). This has also been reflected in the finding that in comparison with normal weight individuals, obese individuals skipped breakfast or lunch more frequently and when they do, a larger dinner meal is consumed (Berg *et al*. 2009). A higher food intake in the morning and afternoon has been associated with lower total energy intake compared to a higher food intake later during day and in the evening as a result of skipping breakfast (De Castro 2004).

Two forms of *night time eating* namely night eating syndrome (NES) and sleep related eating disorder (SRED), may be associated with obesity development (Howell *et al*. 2009). In a review by Howell *et al*. (2009) it was indicated that a definite conclusion that NES causes obesity can not be made at this stage due to a lack of research in this area. However, available evidence points to a possible causal relationship as NES was found to correlate with higher BMIs, the resistance to lose weight on weight reduction programmes and that NES is more prevalent in obese than non-obese individuals (Howell *et al*. 2009). The association between SRED, weight gain and obesity has been confirmed. Both NES and SRED are associated with an inappropriate time of eating when metabolic responses to food intake is different from responses to day-time eating, inappropriate food choices and binging (Howell *et al*. 2009). Individuals with NES eat similar foods during the night as during the day, although they show definite preference for carbohydrate rich foods such as breads and sugars. NES is also associated with being more psychologically distressed and depressed, which may influence food intake (Calugi *et al*. 2009, Olbrich *et al*. 2009). On the other hand, SRED is often associated with frequent sleep walking and unconscious nocturnal eating that consists of specific combinations of foods, inedible substances, dangerous food preparation behaviour and foods high in carbohydrates and fats such as bread, pies, ice cream, chocolate, sweets and peanutbutter (Howell *et al*. 2009).

2.1.2 Physical activity

When considering the other end of the energy balance equation, namely energy expenditure, there is ample evidence that low physical activity levels relate to weight gain and contribute to the development of obesity. In economically active South African adults it has been found that low physical activity levels when not at work and self-reported low or medium physical activity levels significantly predict obesity development (Senekal *et al*. 2003). Low physical activity usually results from a combination of aspects, including no participation in formal exercise or sports, increased engagement in sedentary behaviours especially during leisure time, decreased occupational activity and decreased household activities that include activities undertaken as part of day-to-day

living. Increased sedentary behaviours or physical inactivity accounts for the large documented decreases in energy expenditure in various populations. These include physical passive behaviours such as watching television (TV), computer work, driving a car, talking to friends on telephone instead of walking to them, reading and other inactive daily doings (WHO 2000). Gorden-Larsen *et al*. (2009) found an 8 kg higher weight gain over a period of 15 years in women with no leisure time walking compared to those who walk for 30 minutes a day. Furthermore, several studies have indicated that TV viewing is the dominant leisure time activity for many individuals and the amount of hours of viewing is positively associated with higher BMIs and lower levels of fitness (Swinburn & Shelly 2008). Three possible explanations for this positive relationship have been suggested by Swinburn & Shelly (2008), including 1) TV viewing causes obesity, 2) obesity causes high TV viewing and 3) lower socio-economic status could be responsible for high TV viewing. It is also possible that a vicious cycle between these components exists. The relationship is mediated through the availability of less time for physical activity, reduced resting metabolic rate and increased energy intake (Swinburn & Shelly 2008). The increased energy intake results from eating high-fat and unhealthy foods while watching TV. TV watching also increases exposure to the marketing of energy-dense foods and beverages which may stimulate consumption (WHO 2000, Swinburn & Shelly 2008).

Besides the overall positive effect of regular participation in exercise or sports on energy expenditure and thus weight management, exercise also suppresses hunger. Furthermore, it has been shown that individuals who regularly participate in moderate exercise develop an increased ability to burn fats rather than carbohydrates as a fuel (WHO 2000).

2.1.3 Built-environment

The term, built environment, refers to those elements of an individual's external surroundings that are human-made or modified in comparison to naturally occurring environment elements surrounding individuals (Papas *et al*. 2007). The built environment is considered to be a contributing factor in the development of obesity when it creates a milieu that promotes increased energy consumption and decreased physical activity (Black & Macinko 2008). To have an impact on weight status, an interaction between the built-environmental factors and the individual takes place. This interaction and the outcome thereof may vary for instance between men and women, families, family members, age groups and socio-economic status groups (Papas *et al*. 2007). Papas *et al*. (2007) confirmed that 17 of the 20 studies reviewed by them showed a positive relationship between elements of the built environment leading to increased energy consumption and/or decreased physical activity and obesity development.

The influence of the built environment on physical activity

It is evident from various reviews that modernization and changing societal structures have created an environment that promotes a more sedentary lifestyle (Booth *et al*. 2005, Papas *et al*. 2007, Black & Macinko

2008). Several elements in the built environment and neighbourhood design can affect physical activity levels. These include:

- How individuals get from one place to the other with available roads, transportation systems, bicycle and walking paths;
- Places where individuals spend their leisure time for example available parks and public facilities;
- Places where individuals can exercise such as available gyms and sport clubs; and
- Social values and perceptions including crime, traffic, reputation, aesthetics, litter, trust and capital that impact on individuals as to how they use their neighbourhoods (Black & Macinko 2008).

In current day society the energy expended on various day-to-day living tasks is clearly less than a few decades ago (summarized in Table 2.2).

Table 2.2: Energy expenditure on day-to-day living tasks in current day society compared to a few decades ago.

Day-to-day living task	Current day society	Consequence of current day society factors	A few decades ago
Transportation	▫ Increased car ownership; ▫ Improved public transport systems.	▫ Travels short distances by car, train, bus or other automobiles.	▫ Walked or cycled short and long distances.
Household tasks	▫ Availability of washing machines, tumble driers, vacuum cleaners.	▫ Spends less physical activity on household tasks.	▫ Increased activity on these tasks as machines were not available.
Food preparation	▫ Ready prepared food or ingredients for a dish; ▫ Motorized and labour-saving kitchen equipment.	▫ Spends less physical activity on food preparation.	▫ Food procurement and preparation was more labour intensive.
Occupation	▫ Advancement in mechanization, robotics, computerizations, control systems; ▫ More people in service, clerical, other professional occupations; ▫ Less people in jobs such as forestry, mining and farming.	▫ More sedentary jobs; ▫ Less physically demanding jobs; ▫ Less energy expended even in physically demanding jobs due to improved machinery.	▫ More people in physically demanding jobs. ▫ These occupations were more labour intensive than today.
Electricity and fuels	▫ Increased availability of electricity and other easily available fuels; ▫ Central heating.	▫ Decreased energy expended as thermoregulation is not necessary with central heating; ▫ Decreased activity to keep warm.	▫ No central heating ▫ Collect and prepare fuels such as wood for heating or lighting.
Public places	Automatic time-saving devices such as lifts, elevators, automatic doors	Decrease energy expended.	No such devices Climb stairs
Leisure-time activities	Dominated by watching TV and other sedentary behaviours	Decrease leisure-time activity	Decreased time spent on watching TV

Summarized from WHO 2000 and Popkin 2006.

Other built environmental elements that have been associated with decreased physical activity levels and obesity development include (Booth *et al*. 2005, Papas *et al*. 2007, Black & Macinko 2008):

- Decreased number of recreational facilities near an individual's residence;
- Increased distance from an individual's residence to nearest recreational facility;
- More daily time spent in cars and higher kilometres travelled in a car;
- Living on a highway;
- Living on a street with no sidewalks or with sidewalks on one side only;
- Living in neighbourhoods with:
    - Low walkability;
    - Lower levels of greenery;
    - Increased residence density;
    - Increased population density;
    - Decreased land-use mix (residential, commercial and institutional facilities are not in close proximity of each other, which makes the community less walkable);
    - Increased sprawl index (model that includes residential density, land-use mix, degree of centering and street accessibility);
    - Barriers to physical activity such as: more crime, traffic and undesired topography (steep hills).

The influence of the built environment on availability of food and food intake

The food environment has change dramatically over the past 30 years favouring excessive energy intake and obesity development. It is apparent that food has become increasingly accessible at prices that are generally more affordable (Wardle 2007). This situation can be ascribed to major subsidies on feed grains, livestock research and livestock production (Popkin 2006). Major advances in agricultural technology that have led to improved cultivation techniques, the use of fertilizers, the ability to grow a wider variety of crops that are disease-resistant and can tolerate problems with soil, temperature and insects also contribute to the situation (Cohen 2008). The consequent dramatic increase in livestock and crop yield together with the advances in food technology, production and preservation, product development, transportation and storage, resulted in an increased per capita food availability at lower prices (WHO 2000, Cohen 2008). Technological advancements and major political and economical initiatives also led to the development of high-yield oilseeds and thus high-quality refined vegetable oils, contributing to major reduction in the cost of fats for baking and frying, margarine and salad oils (Popkin 2006).

All these changes in food production led to the availability of food regardless of season, increased accessibility and consequent consumption of animal source foods, highly processed foods and edible oils, with a shift away from fruits, vegetables and whole grains (Popkin 2006, Cohen 2008). It is a matter of concern that location and accessibility to supermarkets and fast-foods restaurants influence consumption of unhealthy and healthy foods and consequently weight status (summarized in Table 2.3).

Table 2.3: The characteristics of the current-day food environment and associated effects on lifestyle practices and BMI

Characteristics of food environment	Association with lifestyle practices and BMI	Reference
▫ Living in neighbourhoods with: - ↑ fast-food restaurants - ↑ convenience stores - ↓ grocery stores - ↓ supermarkets	▫ unhealthy dietary practices ▫ ↑ BMI ▫ ↑ obesity prevalence	Maddock 2004 Morland *et al*. 2006 Inagami *et al*. 2009 Larson *et al*. 2009
▫ ↑ density (number) of fast-food outlets, convenience stores, restaurants and lunchrooms	▫ ↑ accessibility of meals prepared away from home ▫ ↑ weekly visits to fast-food restaurants ▫ ↓ confidence in eating healthy foods ▫ not meeting physical activity goals	Cohen 2008 Li *et al*. 2009
▫ Greater distance required to travel to a supermarket	▫ ↑ BMI ▫ ↓ fruit and vegetable consumption ▫ ↑ unhealthy food in diet	Rose & Richards 2004 Black & Macinko 2008 Inagami *et al*. 2009 Zenk *et al*. 2009
▫ Better access to convenience stores	▫ ↓ fruit and vegetable consumption ▫ ↑ obesity prevalence	Larson *et al*. 2009 Zenk *et al*. 2009

Additionally, chocolates, chips, sweets, soft drinks and a variety of convenience food are now available at easily accessible vending machines or retail outlets that do not typically sell food as their primary business such as video shops, petrol stations and hardware stores. These non-food establishments often stock highly processed and non-perishable foods that are high in sugar and fat and of low nutritive value (Cohen 2008). The fact that food prices in grocery stores and supermarkets are relatively reasonable, also results in a situation where a lower percentage of household income is spent on purchasing foods at these stores. This leaves more money to afford the convenience of buying ready prepared food away from home (WHO 2000). In the United States of America it was found that only 24% of all household food expenses went to food prepared away from home in 1966 compared to 42% in 2006 (Cohen 2008).

What is of further concern is that it is now possible to manufacture food products with virtually any textural, nutritional and tastes properties (WHO 2000). Manipulation of food products have gone to such extremes that it is difficult for individuals to associate visual, textural or taste cues with the nutritional and energy content of meals. With the increasing trend towards consuming convenient pre-packed food and dishes, individuals are literally losing control over the preparation of food and the nutritional content is determined by the manufacturers (WHO 2000). It needs to be borne in mind that the WHO (2000) claim that the food supply is now literally in the hands of a small number of large multi-national companies that are more concerned about the production of tasty cheap foods or food products to ensure financial gains, than the nutritional content and health impact thereof. For instance, processed food products rich in refined grains, added fat and added sugar are usually considerably cheaper per thousand kilojoules and a higher consumption level is reported for these foods compared to more expensive fresh fruits and vegetables (Drewnowski 2007, Bray 2008). The calculated

energy cost of fresh produce such as fruit and vegetables is 10 times higher as that of vegetable oils and sugars (Drewnowski 2009). Therefore, this results in a paradox of ability to spend less but eat more energy, provided that the energy comes from added fat and sugar (Drewnowski 2009).

Finally, it needs to be considered that industrialization at global and national levels has resulted in large-scale urbanization as people are searching for work, a higher income and a better life (WHO 2000, Candib 2007). Urbanization goes hand-in-hand with high levels of unemployment, poverty, increased exposure to cheap, processed foods and less access to home-grown and local vegetables. The resulting diet is high in protein, fat, especially saturated fatty acids, kilojoules and less in complex carbohydrates (WHO 2000, Candib 2007). These dietary changes are referred to as the 'nutrition transition' experienced by urbanization and contributes to obesity development (Kruger *et al*. 2005, Popkin 2006).

Governance

The government of countries also play a role in addressing the problem of obesity as they make final decisions and enforce laws that may influence food intake and physical activity levels (WHO 2000). For instance, the amount of time spent in school curriculums on physical activity and nutrition teaching are prescribed by the Departments of Education. Governments also influence infrastructure by allocating open spaces, previously used for recreational activity, to other purposes such as building apartments and shops. Goverments have the power to regulate food quality and safety together with the labelling and advertising of foods. However, many have failed in these tasks and consequently consumers are badly informed, confused about conflicting messages, and exposed to an environment favouring obesity development (WHO 2000).

2.1.4 Social environment

Marketing and advertising

Food choices are influenced by messages and advertisements conveyed to the public through the media, including television, radio and printed material (WHO 2000). With the commercialization of food production and increased competition between large multi-national food production companies and fast-food restaurants, marketers were employed to increase the sales of their products and thus profit (WHO 2000). Marketing strategies such as "eat as much as you can" or "upsize" portion sizes at many fast-food outlets or restaurants create the impression amoung consumers that they will receive better value for money if making these choices (WHO 2000, Gee *et al*. 2008). Furthermore, substantial advertising campaigns that are very convincing and successful are continuously launched to increase sales (WHO 2000). It has been shown that individuals with a high exposure to these commercials through watching more hours of TV per day eat fast-foods more often than those watching less TV per day (Scully *et al*. 2009). It is evident that messages relating to fast-foods, energy-dense foods, carbonated soft drinks and other unhealthy foods far exceed those of fruit and vegetables (WHO 2000, Rosenheck 2008). At the same time advertisements focused on weight loss products and the very thin female fashion models are rife, contributing to very conflicting messages sent through the media (WHO 2000). It

is important to note that programs aired and advertisements published in developing countries are rapidly shifting from a traditional content to a more modern Westernized content, favouring exposure of these populations to energy-dense foods (Popkin 2006).

Cultural influences

Cultural influences such as religious practices, peer group pressure, social gatherings, status assigned to certain foods and individual lifestyles all affect food selection as well as a person's view of their weight and weight management (WHO 2000, Kruger *et al*. 2005, Candib 2007).

In many industrialized countries thin women symbolize competence, success, control and sexual attractiveness, while obesity represents laziness, self-indulgence and a lack of power. Therefore, women in such countries are inclined to be dissatisfied with their body shape, idealize being thinner and often engage in inappropriate dieting regimes to achieve unrealistic weight goals. However, weight loss following such diets is usually short-lived and is soon followed by weight gain and more attempts to lose weight (WHO 2000). Unsuccessful weight reduction attempts or the inability to maintain weight after initial weight loss could be the starting point of a never-ending cycle of weight gain and dieting. Weight cycling in itself has been associated with subsequent large weight gains (Kroke *et al*. 2002) and an increase in the waist-to-hip ratio in women (Rodin *et al*. 1990). Senekal et al. (2003) have shown that the absence of weigth loss attempts in the previous year was a significant protective factor for the risk of obesity in economically active South Africans.

In South Africa, black women are less likely to perceive themselves as being obese compared to white women, although more black women were actually classified as being obese compared to white women (Puoane *et al*. 2002). This reflects how some cultures, including black South Africans, view fatness in women as a sign of health, prosperity, being attractive, sexually appealing, powerful, a good wife, cook and mother and showing that their husbands are good providers (Puoane *et al*. 2002, Puoane *et al*. 2005, Faber & Kruger 2005). Weight loss or being thin may be associated with being more likely to die, often from a stigmatized disease such as tuberculosis or acquired immuno-deficiency syndrome (Puoane *et al*. 2002, Puoane *et al*. 2005). The body image of Caucasian South African women is more in line with those of women in industrialized countries (Cilliers *et al*. 2006). In general, men from developing and developed countries associate "bigness" or a large body structure and muscularity as ideal. Many men, therefore would not view adiposity to be a problem and would not attempt to lose weight as frequently as women (WHO 2000).

Certain cultures or countries highly prize fitness and vitality and have a general positive attitude towards a healthy lifestyle. Individuals from these cultures or countries engage in considerable amounts of vigorous physical activity during their leisure-time. In contrast, individuals from other cultures or countries practice more sedentary lifestyles as they do not understand the importance and necessity of physical activity in being healthy (WHO 2000).

Similarly, cooking methods and foods eaten may be culture specific with some cultures being traditionally inclined to have a higher fat intake. Food, instead of its nutritive value, has been viewed over many centuries as a means to establish and express relationships between people and as one of the most important components of social gatherings such as birthdays, weddings and religious festivities (WHO 2000). Christakis and Fowler (2007) have observed that obesity spreads through social networks, with friends or siblings from the same gender having the greatest effect on an individual's weight gain than opposite gender friends, siblings or spouses. Within these social networks, normal and overweight children and adults had a higher food intake when eating together with friends or family compared to having a meal with a stranger (De Castro 1994, Salvy *et al*. 2007, Salvy *et al*. 2008, Salvy *et al*. 2009). The possible explanation behind this is that individuals are more likely to attend to their food intake in the company of strangers as they still need to be accepted and make an impression on them. However, the accepted and well-known relationships that exist with family and friends may lead to care-free overconsumption (Salvy *et al*. 2009). Furthermore, an individual may recognize a higher food intake or weight gain seen in friends, partners or siblings as a type of "permission" to also indulge or gain weight as it is the norm within their social network and will thus be accepted by their peers (Christakis & Fowler 2007). This may also explain the fact that being married incurs a risk for a higher BMI and waist circumference in South African Blacks (Malhotra *et al*. 2008).

2.1.5 Socio-economic status and education

When considering the socio-economic status (SES) of an individual, factors such as income, education, occupation as well as place of residence (urban or rural) are usually taken into account. These indicators may influence weight status independently or in combination and therefore studies referring to the effect of SES may include any one or combination of these indicators (WHO 2000).

In developed countries it has been consistently shown that individuals with a lower SES or individuals from households with a lower SES have a greater risk of being obese (Ulijaszek 2007, Brennan *et al*. 2009, Drewnowski 2009). However, in many developing countries, thin adults are stigmatized as being poor while overweight or obesity is viewed as a symbol of affluence (WHO 2000). Therefore, in developing countries more affluent individuals have a higher risk for being obese, while those with a low SES usually have a decreased obesity risk (WHO 2000, Drewnowski 2007, Ulijaszek 2007). In both developed and developing countries the increased obesity risk associated with either low or high SES is more profound in women and not always applicable to males (Drewnowski 2007). It is also now evident that as affluence in some developing countries has grown, the relationship between SES and obesity has changed towards a similar picture seen in developed countries, with low SES being associated with obesity (WHO 2000). Because the population in the current study reflects an urban developed community, the focus of the rest of this section is on the association between SES and weight status in developed countries. The relevant studies have mostly been executed in the United States of America, Canada, Australia, England, France and other European countries (Drewnowski 2009).

Possible explanations for the association between lower SES and obesity in developed countries are related to the fact that these individuals are more likely to consume energy-dense diets that are high in energy, refined grains, sugar, salt, total fats and saturated fats and low in micronutrients and fibre (Mendoza *et al*. 2007, Drewnowski 2007, Drewnowski *et al*. 2007, Black & Macinko 2008, Savage *et al*. 2008, Drewnowski 2009, Monsivais & Drewnowski 2009, Townsend *et al*. 2009). It is further evident that energy-dense diets are less expensive and very palatable, while actually consisting of less food (in weight) when compared to low energy-dense diets (Drewnowski & Darmon 2005, Maillot *et al*. 2007). The lower satiating power of energy dense diets may result in passive overeating (Drewnowski & Darmon 2005, Drewnowski 2007). In a review by Darmon and Drewnowski (2008) it has been shown that individuals with a low SES specifically have higher intakes of white bread, rice, potatoes, organ meats, fried, canned or stewed meat, sausages, fried fish, eggs, full cream milk, animal fats, sugar, sweetened beverages and beer. In contrast, individuals from a higher SES consume more whole grains, higher quality lean meats, fish, low-fat dairy products, nuts, fresh vegetables and fruits and wine (Kirkpatrick & Tarasuk 2003, Drewnowski 2007, Darmon & Drewnowski 2008). Furthermore, a larger variety of fruits and vegetables with more lettuce based salads, melons and berries were consumed by high income households, while low income households preferred less expensive fruits and vegetables such as bananas and potatoes (Drewnowski 2007).

The consumption of energy-dense diets by individuals with a low SES has been associated with higher weight gains over a six year period and higher BMIs (Savage *et al*. 2008). It was also shown that obese individuals, when compared to normal weight individuals, usually consume energy-dense diets (Ledikwe *et al*. 2006, Drewnowski 2007). The purchase of unhealthy, energy-dense foods by individuals with a low SES is inherently driven by the lower cost thereof, but is also linked to the perception that healthy foods are more expensive and unaffordable (Giskes *et al*. 2002, Turrell & Kavanagh 2006). Recent research in South Africa points to the fact that a healthy diet is more expensive than an energy-dense unhealthy diet (Temple et al. 2010; Temple & Steyn 2010). These authors have shown that replacing six commonly consumed energy-dense foods (hamburger, full cream milk, corn flakes, brick margarine, white rice and white bread) with healthy nutrient-dense alternatives amounts to a 10 to 60% increase in cost when compared per 100g food or a 30 to 110% increase when comparing the food per megajoules content. Furthermore, a healthier alternative to a typical South African daily menu of an average adult was calculated to be 69% more expensive. The authors argued that the latter price difference can decrease by half with motivation, education and careful selection of cheaper healthy foods (Temple et al. 2010; Temple & Steyn 2010). However, it has been shown that lower education levels (Monsivais & Drewnowski 2009) and poorer dietary knowledge of many individuals with a low SES contributes to the selection of unhealthy energy-dense foods (Turrell & Kavanagh 2006). In fact, lower education levels alone have been linked to a higher prevalence of obesity when compared to groups with higher education levels. The WHO (2000) claims that individuals with higher education levels are more likely to follow dietary recommendations and adopt other risk-avoidance behaviours than those with lower education levels. Individuals from a high SES are usually thinner and characterized by having an overall better quality diet, higher educational levels and a better awareness of the role of nutrition in health (Drewnowski 2009). In groups from lower SES, lifestyle choices such as smoking,

larger serving sizes of food and reduced physical activity were linked with obesity and poorer health (Brennan *et al*. 2009). However, the association between nutrition knowledge and obesity prevalence is not always consistent. The findings that some individuals with better nutrition knowledge still prefer eating unhealthy food may be linked to the fact that the long-term effect of an unhealthy lifestyle on health outcome is not well understood (WHO 2000).

Within the South African context, Puoane *et al*. (2002) reported that lower BMIs in South African women were associated with lower education levels or no schooling. This might be explained by the fact that these women tend to do more manual labour compared to their schooled counterparts. However, when considering South African women with schooling, the picture is in line with global findings from developed countries namely that women with tertiary education had lower BMIs compared to those with no further tertiary education (Puoane et al. 2002). Puoane *et al*. (2002) speculated that these women may have a better understanding of the impact of body weight on health, resulting in the negative association. However, these authors maintain that body image may play a bigger role and that educated woman may endeavour to control their weight in order to conform to Western ideals of thinness (Puoane *et al*. 2002). As far as South African men are concerned, it is evident that obesity is more likely to occur in those who live in urban areas, are highly educated and are white (Puoane *et al*. 2002). Gender and age-adjusted data of economically active South African adults from Asian, Black, Caucasian and Mixed ancestries confirms that a lower education status (≤ grade 7) is associated with an increased risk for obesity (Senekal *et al*. 2003). However, Malhotra *et al*. (2008) found no association between education status and the risk of being obese in Black urban South Africans.

A recent review indicates that the neighbourhood an individual lives in may also play a role in terms of SES related factors and obesity development. Research consistently shows a link between living in an economically deprived neighbourhood and higher odds of being obese or having higher BMIs (Black & Macinko 2008). Low income neighbourhoods usually have fewer large supermarkets, fewer supermarkets per person, a lower availability of healthy foods such as low-fat milk, high-fibre bread and fresh fruits and vegetables and are often further away from the nearest supermarkets (Black & Macinko 2008, Darmon & Drewnowski 2008, Drewnowski 2009). In contrast, energy-dense foods seem to be more accessible in such neighbourhoods as fast-food outlets, convenience stores and small local vendors are common. Healthy food is thus more difficult to find, more expensive and possibly of lower quality compared to the large variety available in retail supermarkets (Black & Macinko 2008, Darmon & Drewnowski 2008, Drewnowski 2009). Similar findings have been reported in rural areas and townships in South Africa where shops and street vendors only stock full cream milk, high fat meats and snacks, fried foods and few fresh fruits and vegetables (Faber & Kruger 2005, Chopra & Puoane 2003). In contrast, more affluent neighbourhoods generally have lower exposure to fast-food restaurants, but higher access to fresher produce, large supermarkets and restaurants serving better quality and healthier foods (Black & Macinko 2008, Drewnowski 2009). Furthermore, more opportunities for physical activity are generally found in neighbourhoods with high SES whereas people from lower SES neighbourhoods are more likely to be sedentary (Black & Macinko 2008, Drewnowski 2009). These individuals are probably more affected by some

aspects of the built environment such as smaller activity spaces, fewer parks and other recreational facilities, less "walkable" neighbourhoods and a lack of resources and transportation to afford and access physical activity opportunities (Papas *et al*. 2007, Black & Macinko 2008). Furthermore, these residents report that crime, unattended dogs and less trust of neighbours are additional barriers to physical activity (Black & Macinko 2008). As a consequence, low SES individuals were found to watch much more television than those with high SES (WHO 2000).

The changing role of women in modern societies has also influenced family eating habits. With more women entering the job market and returning to work soon after childbirth, less time is spent on shopping, preparing food and doing other household tasks (WHO 2000). While women usually still take responsibility for the health and well-being of their families, many lack the motivation, skills, energy and time to prepare food for their families. This situation together with the fact that working women now have access to their "own money", has contributed to the creation of a market for time-saving, prepared and convenience foods (WHO 2000, Popkin 2006, Ulijaszek 2007). These factors result in an increasing consumption of unhealthy fast foods and other convenience foods, contributing to the rise in childhood and adult obesity that has been observed (WHO 2000).

In summary, a lower income, lower education, minority status, and a higher incidence of poverty are linked to higher obesity prevalences (Drewnowski 2009). Furthermore, obesity in women is generally associated with a low income and education status while this association is less consistent in men (Drewnowski 2009).

2.1.6 Behavioural and psychological factors

Eating behaviour and the specific components thereof including dietary restraint, disinhibition and hunger have been linked to weight gain and obesity development (Bryant *et al*. 2008). The Three-factor eating questionnaire (TFEQ) is the most widely used instrument measuring the three components of eating behaviour. Individuals with a higher disinhibition score are characterized with having a higher tendency to overeat for instance, when food is very palatable, when in the company of someone that overeats, when at social occasions, when having an emotional or personal problem or when stressed (Stunkard & Messick 1985, Bryant *et al*. 2008). A review by Bryant *et al*. (2008) indicates that these individuals prefer high-fat foods, high-fat and high-salt foods, processed meats, sweet fruits and vegetables, and sweet carbonated drinks and have higher intakes of sweets, cookies, ice cream, butter, coffee and alcohol. Higher disinhibition scores have consistently been associated with a higher weight, BMI or weight gain over time (Bryant *et al*. 2008, Chaput *et al*. 2009, Rideout & Barr 2009, Savage *et al*. 2009). Furthermore, obese individuals have higher disinhibition scores than their normal weight counterparts (Bryant *et al*. 2008). These associations were found to be independent of SES, dieting status and previous weight history (Bryant *et al*. 2008).

A higher restraint score is associated with employing specific dieting related strategies to consciously limit food intake in order to control body weight. These strategies include the avoidance of fattening foods, eating smaller

portions, stop eating before reaching satiation and consistently considering the energy content of food in order to control energy intake and to buy low energy containing foods (Bryant *et al*. 2008). It has been reported that a lower restraint score is associated with a higher body weight (Hainer *et al*. 2006, Rideout and Barr 2009) and that decreases in dietary restraint over time were associated with concurrent weight gain (Savage *et al*. 2009). However, the results of studies investigating these relationships are inconsistent (Bryant *et al*. 2008, Savage *et al*. 2009). It has been reported that the combined effect of restraint and disinhibition influence body weight. Therefore, the effect of disinhibition on weight disappears when a high level of restraint eating is present (Hays *et al*. 2002, Savage *et al*. 2009). Individuals with the highest weight have high levels of disinhibition together with the lowest levels of restraint (Hays *et al*. 2002, Dykes *et al*. 2004). Perceived hunger refers to how often hunger feelings are felt and the extent to which these feelings increase food intake. A higher hunger score has been associated with a higher weight (Chaput *et al*. 2009), and weight gain over a four year period (Hays *et al*. 2006). Obese individuals also have a higher hunger score than normal weight individuals (Harden *et al*. 2009).

Other behavioural and psychological traits that have been associated with weight gain and obesity development include having a lower body image, self-esteem and self-motivation and poor coping or problem-solving skills (Byrne 2002). These associations have also been reported in South African population groups. In a sample of South African university students, mainly Caucasian, a higher BMI was significantly associated with a lower self-concept, having more body shape concerns and disordered eating patterns (Cilliers *et al*. 2006). Senekal et al. (2003) have shown that the absence of binging in economically active South African adults decreases the risk of developing of obesity (Senekal *et al*. 2003). Furthermore, various mental and personality disorders including mania, anxiety and depression contribute to the hedonic aspects of overeating and may predict the development of obesity in both men and women (Ahlberg *et al*. 2002, Davis 2009). Psychological explanations of obesity focus on addictive personalities, poor decision-making skills, cortisol-mediated responses to stress, or simply on the seeking of comfort in high-fat foods (Drewnowski 2009).

According to Davis (2009), individual differences in the mesocorticolimbic pathway that have previously been associated with drug addiction, are now also viewed as a possible cause for obesity development. Individuals with a sluggish reward system (reward deficiency syndrome) use addictive substances such as food, as a type of self-medication to increase hedonic capacity. Lower dopamine levels due to lower receptor density in the brain have been associated with reduced mesolimbic brain dopamine signalling and a lower ability to experience natural reward. Consequently, affected individuals compensate by overeating energy-dense foods to increase extracellular dopamine levels. On the other hand, a person with a hypersensitive reward system is also at risk of obesity development as they are more vulnerable to overeat due to their better motivation to consume foods that are palatable and provides a pleasurable feeling. These individuals are characterized by emotional eating, binging, having food cravings and a preference for high-fat foods (Davis 2009).

A personality trait referred to as impulsivity has also been strongly associated with overeating and weight gain as these individuals have a heightened drive for smaller immediate rewards which is not counterbalanced by an

appropriate sensitivity to punishment (Davis 2009). Such individuals usually have poor decision-making skills and will therefore engage in highly fun-seeking or risky behaviours to provide an immediate reward without considering the consequences. In the current food environment that promotes the availability of a variety of highly palatable foods may prove to be problematic if a person is inclined try all the different food options for immediate reward, often resulting in overconsumption and thus very high energy intakes (Davis 2009).

The attention deficit/hyperactivity disorder (ADHD) that is characterized by both impulsivity and reward sensitivity has also been associated with obesity. As is mentioned above, it is claimed that some cases of obesity result from a food addiction similar to drug addiction. According to Davis (2009) these forms of obesity may be more pronounced in individuals with ADHD, who regularly binge. It is suggested that obese individuals should be screened for ADHD when seeking weight loss treatment, as methods to treat ADHD may be used successfully in conjunction with weight loss interventions to treat obesity in such individuals (Davis 2009).

2.1.7 Biological factors

Many biological factors influence weight management and though mostly genetically determined may also be influenced by environmental factors. These include hormonal and neural factors that provide short and long-term signals influencing satiety and feeding activities, the number and size of adipocytes, regional distribution of body fat, resting metabolic rate and absorption of fat from food in the gastro-intestinal tract (WHO 2000). Individuals with a genotype that favours the expression of biological factors in such a way that energy storage is promoted are more vulnerable to develop obesity (WHO 2000). A discussion of all the biological pathways contributing to obesity development is beyond the scope of this review. Relevant biological pathways are discussed in the sections on the genes selected for this research (sections 2.3 to 2.8).

2.2 Genetics of obesity

There is no doubt that genotype contributes to the development of the obese phenotype. Although recent research has significantly contributed to the understanding of the link between genes and obesity (Rankinen *et al*. 2006), the specific genes, mutations and physiological mechanisms involved in the development of obesity have not yet been fully elucidated (Romao & Roth 2008).

The contribution of genetics to obesity development has been clearly indicated by heritability estimates defined as “the fraction of the population variation in a trait (e.g. BMI) that can be explained by genetic transmission” (Loos & Bouchard 2003). Heritability estimates derived from a number of epidemiological studies conducted on twins, families and adopted families have indicated that genetic factors are responsible for the variation in human adiposity from as low as 5% to as high as 90% (Loos & Bouchard 2003, Romao & Roth 2008). According to Loos and Bouchard (2003) the differences in methodology used by these studies mostly explain this wide

range of heritability estimates. The highest heritability levels, with values around 70%, are reported by studies conducted on monozygotic and dizygotic twins (Allison *et al*. 1996, Maes *et al*. 1997, Hjelmborg *et al*. 2008, Lajunen *et al*. 2009, Mustelin *et al*. 2009, Ortega-Alonso *et al*. 2009). The lowest heritability levels with values of 30% or less were seen in adoption studies (Sorensen *et al*. 1992a, Sorensen *et al*. 1992b, Vogler *et al*. 1995), while family studies revealed intermediate levels between twin and adoption studies (Rice *et al*. 1999, Coady *et al*. 2002, Choy *et al*. 2009). The role of genes in obesity development is further supported by studies that report that a family history of obesity predisposes an individual to a two- to three-time higher risk of becoming obese. A family history of severe or extreme obesity (BMI≥45) respectively results in a three- to six-time or a seven- to eight-time higher risk to develop obesity (Loos and Bouchard 2003).

Elucidation of the actual genes involved as well as associated mechanisms has been the focus of research for many decades. As early as 1962, Neel proposed the "thrifty" genotype hypothesis, namely that people who are genetically prone to develop type-2 diabetes have a "thrifty" genotype that is responsible for high levels of food intake and/or low levels of utilization and the consequent development of obesity. According to Neel (1962) the thrifty genotype evolved during the hunter-gatherer era during which food was scarce. Hunter-gatherers had to engage in very high levels of physical activity to obtain food. Periods of food abundance after successful hunting was alternated with periods of greatly reduced food intake and the risk for famine was always present. Consequently, through natural selection, the human biological systems evolved to fight against food shortages, with the body storing any excess energy for the possibility of future food deprivation (Loos & Bouchard 2003, Rosmond 2004, Romao & Roth 2008). Neel (1962) linked the development of diabetes in current times to a quick insulin trigger protection mechanism that limits renal glucose loss that was beneficial for the hunter-gatherer who was repeatedly exposed to feast and famine situations. Bouchard (2007) indicated that "although the original 1962 paper did not specifically focus on energy balance and obesity, the "thrifty" genotype hypothesis has been mostly cited as the source for the notion that the biological predisposition to obesity resides in genetic features that favour thriftiness". Because certain concepts on which the thrifty genotype hypothesis was based, were not confirmed in later years, Neel (1999) suggested that the original thrifty genotype hypothesis needed revision (Neel 1999).

Taking this into account, Bouchard (2007) proposed five new genotype-based hypotheses for obesity development. These were formulated by identifying the genes that are supported by at least five positive association studies with an obesity phenotype, as is summarized in the latest obesity gene map (Rankinen *et al*. 2006). The 22 genes that were identified were subsequently classified according to biological or behavioural traits that may favour obesity development. These five hypotheses are as follows:

1) The thrifty genotype: low metabolic rate and insufficient thermogenesis;
2) The hyperphagic genotype: poor regulation of appetite and satiety and propensity to overfeed;
3) The sedens genotype: propensity to be a couch potato or physically inactive;
4) The low lipid oxidation genotype: propensity to be a low lipid oxidizer;

5) The adipogenesis genotype: ability to expand the complement of adipocytes and high lipid storage capacity.

It must be borne in mind that the phenotypic expression of obesity in most individuals is the result of an interaction between genetic factors and the environment (Chung & Leibel 2008, Marti *et al*. 2008, Romao & Roth 2008). This synergistic relationship between genes and environmental factors means that in the presence of genetic mutations for obesity the severity of the disease is largely determined by environmental conditions (Loos & Rankinen 2005, Chung & Leibel 2008).

Environmental influences on gene expression commence in the uterus during pregnancy. Fetuses may suffer from intra-uterine growth retardation when exposed to prenatal energy deprivation as a result of low maternal energy intakes, resulting in low birth weight. Infants and children exposed to early postnatal undernutrition have been found to have a higher risk to develop obesity later in life (Candib 2007). This phenomenon is also referred to as a "thrifty phenotype", where an undernourished fetus, infant or child responds later in life by accumulating excess energy more effectively compared to other individuals when food is available. It is speculated that early energy deprivation causes certain genes to be permanently "switched on" by the fetus, favouring energy conservation, causing maximum fat storage when the individual is exposed to an abundant food environment later in life (Candib 2007).

After birth, weight gain will most likely occur when individuals who live in a 'restrictive' environment (associated with high physical activity levels and the intake of a diet rich in whole grains, fruits and vegetables and low in fat) migrate to an 'obesogenic' environment (see section 2.1). This has clearly been demonstrated in the Pima Indians, who have a high genetic predisposition for obesity and type-2 diabetes. A lower prevalence of obesity and type-2 diabetes is evident in the Pima Indians residing in their traditional restricted environment of the remote Mexican Sierra Madre Mountains, than their counterparts who live in the 'obesogenic' environment of Arizona in the United States of America (Ravussin *et al*. 1994). The work on the Pima Indians affirms the notion that modern day humans still have the ancient hunter-gatherer genome, which is perfectly suitable in a restrictive environment. The mismatch between the ancient genotypes of humans living in the current-day obesogenic environments explains the high prevalence of obesity (Loos & Bouchard 2003, Rosmond 2004, Loos & Rankinen 2005). However, it is also known that not all individuals living in an obesogenic environment will gain weight. In this environment, individuals who have genetic mutations that promote the development of obesity will gain more weight and probably develop obesity, while those without genetic predisposition for obesity will gain little if any weight (Loos & Bouchard 2003, Loos & Rankinen 2005, Chung & Leibel 2008). Therefore when investigating the causes, pathogenesis or possible treatments for obesity it is always important to consider the gene-environment relationships involved (Loos & Rankinen 2005, Marti *et al*. 2008). The influence of the environment in expressing the obese phenotype has also been illustrated in experimental work in identical twins. Bouchard *et al*. (1990) provided 12 male monozygotic twin pairs with their energy requirement for weight maintenance plus a surplus of 4200 kJ/ day for 100 days. The weight gain of two

individuals forming a twin pair was found to be similar. However weight gain experienced by each of the 12 twin pairs was significantly different, indicating a genetic effect. Similar results were reported in a study during which seven pairs of young adult male monozygotic twins completed an exercise regime over 93 days to create a negative energy balance (Bouchard *et al*. 1994). The two individuals forming a twin pair lost the same amount of weight following the exercise regime. However, when comparing the seven twin pairs, some twin pairs lost more weight than other twin pairs. The amount of weight gained or lost is therefore dependent on a predisposition that appears to be largely inherited (Bouchard *et al*. 1994).

According to Loos and Bouchard (2003) the genetic contribution to obesity can be classified as follows:

- Genetic obesity:

  Includes monogenic cases of obesity i.e. single-gene obesity and Mendelian obesity (see section 2.2.1). These individuals will develop obesity with the environment only playing a permissive role in the severity of the phenotypic expression.

- Strong genetic predisposition:

  Includes polygenic cases of obesity i.e. multiple gene mutations, each having a small effect in causing obesity (see section 2.2.2). These individuals are likely to be overweight in an environment that does not favour obesity development, while they become obese or severely obese in an obesogenic environment.

- Slight genetic susceptibility:

  Also includes polygenic cases of obesity (see section 2.2.2). However, these individuals are likely to be normal weight or slightly overweight in a restrictive environment, while they become overweight or obese in an obesogenic environment.

- Genetically resistant to obesity:

  These people remain normal weight in a wide range of obesogenic conditions.

2.2.1 Monogenic forms of obesity

Single-gene disorders

In rare cases of human obesity the presence of a single genetic mutation in one particular gene results in the development of obesity independent of environmental factors (Loos & Bouchard 2003, Farooqi & O'Rahilly 2005, Mutch & Clement 2006). According to the latest version of the Human Obesity Gene Map (Rankinen *et al*. 2006), 176 human obesity cases due to single-gene mutations in 11 different genes have been reported (Table 2.4). It is clear that the majority of the monogenic obesity cases are attributed to genetic mutations in the *MC4R* gene (143 different cases) (Rankinen *et al*. 2006). The presence of only one of the possible 51 genetic mutations reported to date in this gene (see Table 2.4), will cause obesity. The phenotypic expression of these single-gene disorders is usually severe and has an early onset. For instance, the six cases identified with a mutation in the leptin gene were all homozygous for the mutation and suffered from severe hyperphagia, hypogonadotrophic hypogonadism and morbid obesity from a young age. These symptoms also presented in patients homozygous

for the leptin receptor gene mutation, however the loss of leptin receptor function results in a more severe phenotypic expression than the loss of leptin. In addition, those with the leptin receptor mutation also experience significant growth retardation, hypothalamic hypothyroidism and massive obesity soon after birth. The heterozygous relatives of these monogenic obesity cases also had a higher than expected prevalence of obesity and a higher percentage of body fat compared with ethnicity-matched controls (summarized in Loos & Bouchard 2003).

Table 2.4: Monogenic forms of obesity in the form of single-gene disorders

	Gene	Gene name	Number of defects	Number of individual cases
1	*CRHR1*	Corticotropin-releasing hormone receptor 1	1	1
2	*CRHR2*	Corticotropin-releasing hormone receptor 2	1	3
3	*GPR24*	G-protein-coupled receptor 24	2	3
4	*LEP*	Leptin	2	6
5	*LEPR*	Leptin receptor	2	3
6	*MC3R*	Melanocortin 3 receptor	1	2
7	*MC4R*	Melanocortin 4 receptor	51	143
8	*NTRK2*	Neurotrophic tyrosine kinase receptor type 2	1	3
9	*POMC*	Proopiomelanocortin	3	8
10	*PCSK1*	Proprotein convertase subtilisin/kexin type 1	2	2
11	*SIM1*	Single-minded homolog 1	2	2

Adapted and revised from Rankinen *et al*. (2006) and Pérusse *et al*. (2005)

Mendelian disorders

Mendelian disorders, which are also referred to as syndromic obesity, differ from single-gene disorders because obesity is only a clinical manifestation and not the dominant feature of the condition, as is the case in single-gene disorders (Loos & Bouchard 2003, Mutch & Clement 2006). According to the latest human obesity gene map, 50 loci related to Mendelian syndromes relevant to human obesity have been mapped to a genomic region (Rankinen *et al*. 2006). Causal genes or strong candidates have been identified for most of these syndromes. The Mendelian disorders occur as either autosomal recessive or triallelic/digenic or autosomal dominant or X linked (Mutch & Clement 2006, Rankinen *et al*. 2006). These disorders include among others the Alstrom syndrome, Albright hereditary osteodystrophy, Bardet-Biedl syndrome, Cushing's syndrome, Familial partial lipodystrophy (Dunnigan), Prader-Willi syndrome (PWS), Wilson-Turner syndrome and Insulin resistance syndromes (Rankinen *et al*. 2006). Of these, the PWS is the best characterized and most common with an estimated prevalence of 1 in 25 000. PWS is characterized by obesity, reduced fetal activity, muscular hypotonia at birth, short stature, hypogonadism, mental retardation, small hands and feet and hyperphagia that usually emerges between 12 and 18 months. The syndrome is caused in most cases (70%) by a deletion or disruption of several genes on the proximal long arm of the paternal chromosome 15 (15q11–q13). The symptoms of other syndromes like the Albright hereditary osteodystrophy include obesity, short stature, brachydactyly, subcutaneous calcifications, mental retardation in some cases, hypocalcaemia, elevated serum parathyroid hormone (PTH) levels and parathyroid hyperplasia. The Bardet–Biedl syndrome (BBS) is characterized by obesity, mental retardation, pigmentary retinopathy, polydactyly and hypogenitalism. The prevalence of BBS is 1 in

160 000 according to British studies, 1 in 13 500 in the Middle East due to consanguinity (Loos & Bouchard 2003, Mutch & Clement 2006).

According to Schadt and Lum (2006) obesity due to currently known monogenetic defects is not due to a slower metabolism, but rather due to impaired satiety, affecting the function of appetite control centres in the brain. This affirms that human food intake is controlled by powerful biological signals and is not an entirely voluntarily controllable phenomenon. In monogenic obese cases these basic signalling mechanisms are severely disrupted, which makes it impossible for such individuals to overcome the drive to eat (Farooqi & O'Rahilly 2007).

Although the identification of these genes and cases will not help to combat the global problem of obesity due to low prevalence, they have contributed significantly to the understanding of physiological regulatory pathways of appetite and energy homeostasis (Loos & Bouchard 2003). Additionally, applying this knowledge of the underlying genetic defects in these syndromes contributes to more effective genetic counselling and the development of mechanism-directed pharmacotherapy or antagonists for future treatment or prevention of obesity in these patients (Loos & Bouchard, 2003, Farooqi & O'Rahilly 2007, Romao & Roth 2008). The discovery and understanding of these genetic disorders have also contributed to destigmatization of human obesity and allowed it to be recognized as a biomedical disorder and not simply seen as a moral weakness (Farooqi & O'Rahilly 2007).

2.2.2 Polygenic (common) forms of obesity

Mendelian obesity syndromes and single-gene disorders explain only a small fraction of the global obesity problem (Farooqi & O'Rahilly 2007). The magnitude of the current obese population develops obesity due to polygenic obesity. In this more common form of obesity individuals may have a number of mutations in multiple genes, with each mutation having a minor effect on obesity development. For the phenotypic expression of obesity a combination of these mutations could lead to pronounced weight gain in the presence of an obesogenic environment (Bouchard 2007).

To identify the specific genes involved in polygenic obesity in humans, three approaches can be considered (Loos & Bouchard 2003):

- Candidate gene approach: Research is conducted on candidate genes that are identified based on current understanding of the pathophysiology of obesity as well as possible role of specific genes in the functioning of biochemical pathways related to energy balance regulation or adipose tissue biology (Farooqi & O'Rahilly 2007, Loos & Bouchard 2003).
- Genome-wide linkage scans: These scans are executed to identify specific regions in the chromosomes (referred to as QTLs) and eventually genes in these QTLs that may be linked to the development of obesity (Farooqi & O'Rahilly 2007, Loos & Bouchard 2003).
- Tissue-specific gene expression profiles: These profiles are compared between lean and obese individuals.

According to the latest human obesity gene map, the number of QTLs for obesity-related phenotypes is 253 generated from 61 genome-wide scans. A total of 52 of these genomic regions are supported by two or more studies. From candidate gene studies, 426 positive associations with obesity related phenotypes have been reported for 127 candidate genes. Of these, 22 candidate genes have been positively associated with obesity-related phenotypes in at least **five** studies as identified by Rankinen *et al*. (2006). A further 10 genes have shown positive associations with obesity-related phenotypes in at least **four** studies (Rankinen *et al*. 2006).

Genes selected for investigation in this study (see Chapter 3, Selection of genes, Section 3.4) include *FABP2*, *INSIG2*, *FTO*, *ADRB3*, *ADRB2* and *GNB3*. A detailed discussion of these genes is presented in the following sections of the literature review of which a large component involves summaries of association studies in which the associations between the specific polymorphism in question and BMI, obesity, health and lifestyle indicators were investigated. For each polymorphism the association studies are summarized in a number of Tables. The Tables that focus on BMI and obesity associations provide detail on the study-designs, samples sizes, ethnicity, age specification (e.g. children, adolescents, students, specific adult age range) if other than adult (>18 years), gender (if gender was investigated specifically) and disease states of subjects if specified. This information is not repeated in the Tables focussing on the association between the polymorphism and body fat distribution and content as well as health indicators as it can be derived from the BMI/obesity association tables.

It is evident from the reviewed literature in the following sections that the results of the association studies are often conflicting. Heid *et al*. (2009) indicated between-study heterogeneity of as much as 41% in a recent meta-analysis that included 34 studies involving obesity risk and the *INSIG2* rs7566605 polymorphism. This measure of heterogeneity decreased to 11% when only general population based studies were included in a sub-analysis (Heid *et al*. 2009). The work by Heid *et al*. (2009) points to the fact that a wide range of factors should be accounted for/considered when interpreting the results of association studies. For these reasons a brief perspective of the factors that need to be considered in the interpretation of association studies and thus the information presented in the following sections on the abovementioned genes, is provided below.

A major factor to consider is the different study designs used in association studies, including for example case-control, population-based, family-based linkage and cohort designs. Andersson *et al*. (2009) noted that specific study design aspects that need to be considered include sample size, power estimates, population stratification, genome wide association vs. candidate/pathway approach, Hardy Weinberg Equilibrium, genotyping success and errors, frequency of studied genetic variants, functionality of the genetic variants and replication in independent data sets.

It is clear from the information in the sections that follow that sample sizes vary from less than 50 subjects to large samples or meta-analyses that included more than 30000 subjects. It is generally understood that the power to detect statistical significance improves considerably with larger sample sizes, which may thus explain conflicting results between small and larger studies (Andersson *et al*. 2009, De Krom *et al*. 2009). Although the

results of meta-analyses are deemed more conclusive of associations, such analyses are not available for all associations investigated in this review, or if available, included mixed ethnicities without subgroup analysis (Souza *et al*. 2008). The selection criteria used in meta-analyses may also result in the exclusion of several studies that may affect the association outcome and must thus be considered within that context.

As far as race/ethnic factors are concerned it is recognized that genotype and allele frequencies may vary between populations from different ethnicities or geographical regions (Tiwari *et al*. 2008). Population stratification i.e. when the sample consists of subjects with different race/ethnic background and thus different allele frequencies, might influence the results of studies (Andersson *et al*. 2009). If not taken into account, over- or underestimation of associations can occur (Andersson *et al*. 2009), which might be responsible for the lack of replication in many association studies (Lewis 2002). The possibility that this problem may be prevalent is supported by the facts that race/ethnic composition of samples are inadequately described and that the selection criteria for inclusion are not appropriately explained in many studies (Fullerton *et al*. 2010).

Genotyping errors can be suspected when frequencies are not in Hardy-Weinberg Equilibrium (HWE). However, deviations from HWE can also indicate that the sample consists of a heterogeneous population or in cases it can indicate a true genetic effect or strong association with the disease state. Therefore, the genotype frequencies of control or population-based samples (if selected from large populations where random mating is assumed) should be in HWE, whereas samples consisting of only cases may deviate from HWE (Lewis *et al*. 2002, Andersson *et al*. 2009).

The statistical analyses used in association studies vary considerably and may account for much of the variation in reported results. The different statistical tests and models used often include either one of or a combination of the following: 1) t-tests or linear models that provide indication of association; 2) classification tests that are used to plot the proportion of positive test results among cases (indication of true positive fraction) and controls (indication of false positive fraction) on a receiver operating characteristic (ROC) curve and measuring the area under the curve to provide a guarantee of effective discrimination between cases and controls; and 3) logistic regression tests that predict the risk of disease (Jakobsdottir *et al*. 2009). Furthermore, in these tests different genetic models can be used to investigate the association with a polymorphism, including the genotype (comparing the three genotypes), additive (numerically coded as 0, 1, 2 minor alleles), recessive (mutant homozygous genotype is compared to the carriers of the wild-type allele) or dominant (wild-type homozygous genotype is compared to the carriers of the mutant allele) models (Lewis 2002). It should further be acknowledged that multiple comparisons are often used in genetic association studies, which increase the likelihood of a significant association occurring once in every 20 tests. Andersson *et al*. (2009) summarised three approaches from the literature that can be used to discriminate between false positive and true associations. The possibility that the significant results generated are true associations may be strengthened by 1) recurrence of an association in several case-control sets within a study, 2) the replication of an association in one or two

independent studies and 3) when a functional justification of the polymorphism in the disease biology exists or evidence that the polymorphism is in LD with the functional polymorphism (Andersson *et al*. 2009).

Other factors such as gender, age and level of adiposity of subjects included, as well as the inclusion or exclusion of specific disease states or metabolic conditions or controlling for these confounding factors may also have a profound impact on the results generated (Hainer *et al*. 2008). It is known that the risk for chronic diseases increases with age due to the longer lifetime exposure to risk factors (Joost *et al*. 2007). Therefore, conflicting results could be obtained from samples with different age categories. It is also interesting to note that despite the fact that many studies indicate that data were controlled for confounders, the actual factors controlled for vary across studies (such as age, gender, smoking status, BMI, alcohol intake, disease state etc.), with the reasoning behind controlling for specified factors mostly not mentioned and the uncontrolled data either being presented with the controlled data or not presented or mentioned at all. For instance, associations with the FABP2 Ala54Thr polymorphisms were tested in some samples consisting of males and females without controlling for gender (Lei *et al*. 1999, Canani *et al*. 2005, Takakura *et al*. 2005, Martinez-Lopez *et al*. 2007), while others adjusted for gender (Stan *et al*. 2005, Vimaleswaren *et al*. 2006, De Koning *et al*. 2008, Tavridou *et al*. 2009) or analyzed males and females separately (Duarte *et al*. 2003, Nakanishi *et al*. 2004, Pollex *et al*. 2006). This may also contribute to disparity in findings.

It should also be borne in mind when interpreting association studies that for complex, multifactorial, polygenic phenotypes such as obesity several gene-gene and gene-environment interactions are involved in the development of the disease and secondary complications of the disease (Joost *et al*. 2007). The end-products of a specific biological pathway and consequent phenotype are governed by a combination of genes as well as upstream or downstream effects of the polymorphisms in these genes (De Krom *et al*. 2009). Therefore the results of studies investigating one polymorphism in such a pathway within a particular context may find different results than studies that consider various polymorphisms as well as interactions with various environmental factors.

In the sections to follow a summary of the relevant associations is provided in each sub-section. These summaries also reflect disparate outcomes for a number of the associations investigated, which could be attributed to any one or combination of the above mentioned factors. It is very important to consider all these factors in reviewing available literature, however it was considered to be beyond the scope of this review to provide an in depth analysis and explanation for disparities reported for all these associations. For instance Fullerton *et al*. (2010) recently reported on the ambiguity in the explanation and generalisation of findings when considering only one of the mentioned factors that may influence the results of association studies namely population description (race/ethnicity/geographical area) in 80 published studies that investigated the association between only one polymorphism, the PPARγ Pro12Ala polymorphism and Type 2 diabetes and obesity.

2.3 The Fatty acid binding protein 2 (*FABP2*) gene

2.3.1 The *FABP2* gene

A human cDNA probe was used in somatic cell hybrids by Sparkes *et al*. (1987) to locate the human *FABP2* gene on chromosome four. The same probe was then used to assign *FABP2* to chromosome position 4q28-q31 using fluorescent *in situ* hybridization analysis (Sparkes *et al*. 1987). *FABP2* has a total length of 4914 bp and contains four exons (103 or 128, 173, 108, and 312 bp) separated by three introns (Sweetser *et al*. 1987, NCBI 2009).

2.3.2 The FABP2 protein

FABP2 encodes a 131 amino acids protein referred to as the intestinal fatty acid binding protein (I-FABP or FABP2) that was first isolated and characterized by Ockner and Manning (1974). FABP2 is a member of the family of fatty acid binding proteins (FABPs) that can be divided in two main groups, namely those related to plasma membranes ($FABP_{PM}$) and the cytoplasmic proteins ($FABP_C$) (Chmurzynska 2006). The $FABP_C$ are all small intracellular proteins found in the cytoplasm of many tissues throughout the human body (Ordovas 2007, Karsenty *et al*. 2009). Nine distinct $FABP_C$ members have been identified and named based on the major tissue of expression and chronological order of detection (Ordovas 2007, Karsenty *et al*. 2009). The nomenclature originally used was the first letter of the tissue of expression added to the abbreviation FABP, for example intestinal-type (I-), heart-type (H-), brain-type (B-), epidermal-type (E-), liver-type (L-), ileal-type (Il-), myelin-type (M-), testis-type (T-) and adipocyte-type (A-FABP). However, since it has become evident that the expression of a particular FABP type is not always restricted to only one tissue, but may actually occur in many different tissues, the use of a numerical naming system, based on the order of detection, was found to be more functional (Chmurzynska 2006, Ordovas 2007).

In contrast to many of the other $FABP_C$, *FABP2* is only expressed in the fully differentiated columnar absorptive epithelial cells of the small intestine where it is found in abundance (Sweetser *et al*. 1987, Cohn *et al*. 1992; Besnard *et al*. 2002). It has been indicated that the intestinal expression of *FABP2* mRNA is under dietary control (Ockner & Manning 1974) and also regulated by hormones and transcription factors, such as the peroxisome proliferator-activated receptors (PPARs) (Glatz *et al*. 1995, Bernlohr *et al*. 1997). For example, FABP2 concentration was found to be greater when fed a high fat diet than a low fat diet, greater in the jejunum (primary site for fatty acid absorption) than in the ileum, and greater in the villi than in crypt cells (Ockner and Manning 1974).

FABP2 shares a similar tertiary structure with the other $FABP_C$ consisting of 10 anti-parallel β-strands that form two β-sheet structures that in turn form a β-barrel containing a single ligand binding site (Figure 2.1). The cavity

of the β-barrel is filled with water, lined with hydrophilic and hydrophobic amino acids and closed at the one end by two α-helices (Zhang *et al*. 1997, Weiss *et al*. 2002).

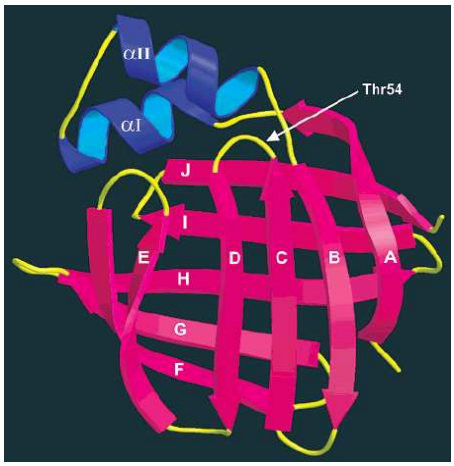

Figure 2.1: The structure of the FABP2 protein. The photo indicates the 10 β-strands (marked A to J), the two α-helices and the position of the *FABP2* Ala54Thr polymorphism (from Zhang *et al*. 2003).

The amino acid sequences of the nine $FABP_C$ vary considerably and are between 17.2% (identity between Il-FABP and M-FABP) to 66.7% (identity between H-FABP and B-FABP) identical to each other. Based on these percentages of amino acid homology between the different $FABP_C$, they are divided into three groups: (1) L- and Il-FABP, (2) H-, B-, E-, M-, A and T-FABP and (3) FABP2 (I-FABP). The members within the same group have similar specific ligand binding abilities (see next section 2.3.3) (Chmurzynska 2006).

2.3.3 Physiological function of FABP2

FABP2 and all the other $FABP_C$ are transport proteins that bind specifically to long chain fatty acids or other lipophilic ligands (Chmurzynska 2006, Ordovas 2007, Karsenty *et al*. 2009). All proteins in group one (grouping introduced in the previous section) bind to ligands such as bile salts, cholesterol and heme, while group two proteins additionally bind fatty acids, retinoids and eicosanoids (Chmurzynska 2006). FABP2 (group three) binds to fatty acids (Chmurzynska 2006) and non-fatty acid lipophilic drugs (Velkov *et al*. 2005). The unique hydrophilic and hydrophobic lining of FABP2's β-barrel cavity results in a high binding affinity for both saturated and unsatured long-chain fatty acids (Baier *et al*. 1995, Baier *et al*. 1996, Weiss *et al*. 2002, Rajabzadeh *et al*. 2003). This fatty acid binding potential of FABP2s together with the fact that they are only expressed in the enterocyte of the gastro-intestinal tract have lead to the hypothesis that they are mainly involved in the uptake and intracellular transport of dietary fatty acids (Weiss *et al*. 2002, Cianflone *et al*. 2008). After digestion of dietary lipids, the digestion products are presented to the brush border membrane of the enterocytes in the small intestine for uptake through a passive- or carrier-mediated process (Chmurzynska 2006). After uptake, the hydrophobic fatty acids face an aqueous environment inside the cytoplasm of enterocytes and rely on a

transport protein to survive (Weiss *et al*. 2002). FABP2 functions inside the enterocytes to bind to these fatty acids and transports them from the brush border membrane to the endoplasmic reticulum (ER) (Cianflone *et al*. 2008). The fatty acids are then released at the site of the ER where the majority are reesterified with glycerol-3-phosphate to form triglycerides and packed into chylomicrons for delivery to peripheral tissues in the circulation (Hegele 1998, Tso *et al*. 2006, Cianflone *et al*. 2008).

However, the exact physiological functions of FABP2 and the other $FABP_C$ are not yet fully elucidated (Helwig *et al*. 2007, Montoudis *et al*. 2008). Various functions including the modulation of intracellular lipid metabolism, modulation of enzyme activity (e.g. lipoprotein lipase and hepatic lipase) and lipid-mediated signal transduction, cell growth and proliferation, indirect regulation of gene expression and control of ligand availability have been proposed, investigated and in some cases ascribed to different $FABP_C$ (Veerkamp & Maatman 1995, Weiss *et al*. 2002, Chmurzynska 2006, Ordovas 2007, Wolfrum 2007). It has been proposed that the $FABP_C$ have the ability to increase the speed of intracellular fatty acid uptake by increasing their rate of dissociation from plasma membranes and enhancing their aqueous solubility inside cells (Weiss *et al*. 2002, Chmurzynska 2006). Furthermore, $FABP_C$ may protect fatty acids against potential detergent-like effects from cellular solutes, but also protect the enterocyte from the cytotoxic effects of free fatty acids (Weiss *et al*. 2002). FABPs may also contribute to the maintenance of membrane integrity by protecting cells from excess free fatty acids (Ordovas 2007). Their involvement in the regulation of gene expression entails the intracellular transport and delivery of regulatory lipids to the nuclear receptors of the PPAR family situated inside the nucleus of cells and enhancing the activation of the PPAR receptors (Weiss *et al*. 2002, Chmurzynska 2006, Wolfrum 2007).

In vivo and *in vitro* experiments have supported some of these proposed functions of FABP2. *In vitro* experiments have shown that Fabp2 transports long-chain saturated fatty acids (SFA) and unsaturated fatty acids (UFA) in Caco-2 cells but incubation with palmitic acid (SFA) resulted in a greater lipid transport compared to oleic acid (UFA) (Baier *et al*. 1996). In one of the more recent *in vitro* experiments, Karsenty *et al*. (2009) have shown that the addition of a fluorescent fatty acid analogue to transfected Cos-1 cells with the wild-type FABP2 resulted in complete colocalization of the FAs and FABP2s in cytoplasmic and perinuclear regions and in cytoplasmic clusters. In the cytoplasmic region, it was shown that the target organelles for FABP2 bound to a FA are not only the ER but also the mitochondria and Golgi apparatus. This was not observed in the vector only cells or the Cos-1 cells transfected with a variant *FABP2* without the α-helical domains. These results indicate that FABP2 binds to long-chain FAs and transports them to specific intracellular organelles and the perinuclear area (Karsenty *et al*. 2009). A possible explanation for this was provided by Montoudis *et al*. (2008) who transfected normal human intestinal epithelial cells (HIEC-6) with cDNA to overexpress FABP2 and compared this with cells treated with an empty vector. They have found that FABP2 overexpression stimulates a higher FA penetration in the mitochondria and higher mitochondrial β-oxidation rates. Furthermore, FABP2 induce the expression and activity of catalytic enzymes necessary for β-oxidation such as the carnitine palmitoyltransferase 1, a critical enzyme controlling the entry of fatty acid (FA) into mitochondria, and increased activity of 3-hydroxyacyl-CoA dehydrogenase, a mitochondrial β-oxidation enzyme. At the same time, it was also indicated

that lipogenesis might be reduced due to the lower gene and protein expression of key enzymes, FA synthase and acetylcoenzyme A carboxylase 2 (Montoudis *et al*. 2008).

The importance of FABP2 and FA colocalization in the perinuclear area (Karsenty *et al*. 2008) was further illustrated by its involvement in the modulation of nuclear receptors by increasing the gene expression of PPARα and PPARγ (Montoudis *et al*. 2008). A possible role in cholesterol metabolism has also been highlighted. FABP2 increases mRNA transcripts, protein expression and activity of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis. Furthermore, FABP2 increases the gene and protein expression of ABCA1 and the protein mass of ABCG5, which functions as an efflux pump in exporting cholesterol out of absorptive cells. In summary, these authors suggest a function for FABP2 in intestinal cholesterol transport and FA delivery to the mitochondria for β-oxidation. Furthermore, they hypothesized that a deficiency of FABP2 may result in increased cholesterol absorption, prevention of FA oxidation and consequently higher blood lipid levels (Montoudis *et al*. 2008).

Although extensive accumulation of FABP2 in the perinuclear area (Karsenty *et al*. 2009) together with its involvement in increasing the expression of nuclear receptors (Montoudis *et al*. 2008) was observed, FABP2 has never been found within the nucleus of cells. Therefore it is still unknown whether the FA actually enters the nucleus, bound or unbound to FABP2, to participate in the regulation of gene expression as seen in other $FABP_C$ (Karsenty *et al*. 2009).

In vitro studies have shown that FABP2 may not be essential for dietary fat uptake from the small intestine in knock-out mice containing no *FABP2* (*Fabp2*$^{-/-}$ mice) (Vassileva *et al*. 2000, Agellon *et al*. 2007). It is known that besides FABP2 expression in the entire intestine, two other $FABP_C$ namely the L-FABP and Il-FABP are also expressed in the proximal and distal small intestine respectively. Therefore, it has been hypothesized that in the intestine of *Fabp2*$^{-/-}$ mice, the expression of L- and Il-FABP is consequently increased to ensure a consistent total pool of FABPs and therefore no change in fatty acid uptake are observed (Vassileva *et al*. 2000, Agellon *et al*. 2007). Although, *Fabp2* knockout does not produce a detrimental effect on dietary fat absorption, it has been shown that in comparison to male wild-type mice, the male *Fabp2*$^{-/-}$ mice developed hepatomegaly on a high fat (Agellon *et al*. 2007) and a high fat and high cholesterol diet (Vassileva *et al*. 2000). Male *Fabp2*$^{-/-}$ mice also gained more body weight and developed insulinemia and elevated TG levels regardless of dietary fat content consumed (Vassileva *et al*. 2000), although Agellon *et al*. (2007) did not find these effects. In females no difference in weight gain was observed when feeding a low fat diet. However when fed a high-fat diet, female wild-type mice gained more body weight and had higher hepatic fat levels compared to female *Fabp2*$^{-/-}$ mice (Vassileva *et al*. 2000). Aged *Fabp2*$^{-/-}$ female mice showed higher plasma insulin levels and better tolerance to glucose challenge than wild-type mice (Agellon *et al*. 2006). These results might indicate a gender-specific effect, namely that in females FABP2 is important to transport fatty acids and contribute to increased fatty acid uptake when consuming a high fat diet resulting in weight gain and insulin resistance. However in males the findings seen in knock-out mice may explain the hypothesis put forward by Montoudis *et al*. (2008) based on their

experimental findings. In other words, a deficiency in FABP2, as seen in knock-out mice, may result in higher cholesterol absorption, higher blood cholesterol and TG levels (Montoudis *et al*. 2008) and this may contribute to the development of hepatomegaly as found in knock-out mice (Vassileva *et al*. 2000, Agellon *et al*. 2007). Montoudis *et al*. (2008) highlighted that no *in vivo* or *in vitro* function can definitely be ascribed to FABP2 and therefore its specific role in the enterocyte is still under investigation.

In summary, although various physiological functions for the FABP2s have been put forward, the specific role thereof in the enterocyte is still under investigation. It is clear that the FABP2s bind to FAs in the cytoplasm of enterocytes and transports them to their sites of utilization. In the mitochondria and nucleus their involvement was shown by increased β-oxidation and nuclear receptor expression respectively. Delivery to the ER might indicate an involvement in chylomicron formation and delivery of FAs to the circulation. They are also involved in intestinal cholesterol transport and cholesterol biosynthesis. A specific gender effect is also possible with females gaining weight and being more insulin resistant as a result of *FABP2* expression, while lower blood and liver lipid levels are found in males possibly due to increased intestinal β-oxidation. Although the functions of the FABP2s are definitely not fully understood it is clear that they play a role in dietary fat assimilation, lipid metabolism and blood lipid profile. These possible functions warrant the consideration of the involvement of these proteins in obesity development, insulin resistance and an abnormal blood lipid profile favouring cardiovascular diseases.

2.3.4 The *FABP2* Ala54Thr polymorphism

A mutation in exon-2 of *FABP2* produces a G to A transition at codon 54 resulting in an amino acid substitution from alanine (Ala) to threonine (Thr) (Baier *et al*. 1995). Amino acids 54 and 55 are situated in a critical tight turn of the tertiary structure of FABP2 where a major conformational adjustment takes place between a free FABP2 and a FABP2 bound to a long-chain fatty acid. When a long-chain fatty acid binds to FABP2 an important position shift between these two residues takes place (Baier *et al*. 1995, Zhang *et al*. 1997). Therefore a change in the specific amino acids at codon 54 or 55 could affect the structure, overall stability or ligand binding potential of FABP2 (Baier *et al*. 1995). A comparison of the three dimensional structures of an Ala54 containing FABP2 and a Thr54 FABP2 reveals that these two proteins are highly homologous (Zhang *et al*. 2003). It appears that only minor local structural changes within the portal region might affect the ligand binding potential. These changes include the possible formation of one additional hydrogen bond across the portal, between the side chains of Thr54 and Asn35, adding stability to the portal. This bond cannot be formed between the Ala54 and Asn35 amino acids. Furthermore, the Thr amino acid has a larger side chain in comparison with the Ala amino acid at residue 54 of FABP2, which may in part block the open end of the portal region. Therefore, Zhang *et al*. (2003) hypothesized that these changes may cause the bound ligand to remain locked inside the protein for a longer period of time. It was also concluded that the different ligand binding capacities of the two FABP2 variants do not originate from direct contact of the bound FA to the different amino acids at position 54, but rather due to changes described above (Zhang *et al*. 2003).

Several *in vivo* and *in vitro* studies have illustrated the functionality of the Ala54Thr polymorphism. *In vitro* studies show that the Thr54 containing FABP2 has a two-fold greater binding affinity for dietary long-chain fatty acids (Baier *et al*. 1995) and is associated with increased transport of fatty acids into enterocytes (Baier *et al*. 1996, Levy *et al*. 2001). In contrast, Kim *et al*. (2001) did not observe any difference in intestinal uptake of FAs when comparing the *FABP2* Thr54Ala genotypes in humans and concluded that the *FABP2* Thr54Ala polymorphism does not enhance intestinal uptake of fatty acids. *In vitro* studies further show that the Thr54 containing FABP2 is associated with increased ApoBI and increased lipid synthesis, specifically with increased TG and phospholipid esterification and TG secretion (Baier *et al*. 1996, Levy *et al*. 2001). Consequently, elevated postprandial chylomicron concentrations were observed in the Thr54 containing protein compared to the Ala54 containing protein in a cellular enterocyte model with Caco-2 cells (Baier *et al*. 1996) and in human intestinal explants (Levy *et al*. 2001). These observations have been confirmed by *in vivo* studies showing an increased lipid profile after ingestion of mixed meals or oral fat tolerance tests (summarized in Section 2.6.9, Table 2.7). Furthermore, in humans Thr54 allele carriers have higher fasting lipid oxidation rates (Baier *et al*. 1995, Kim *et al*. 2001) and 60% higher lipid oxidation rate four hours after an OFTT (Weiss *et al*. 2007).

Although discrepancies in results are evident, available evidence does point to the fact that the presence of the mutant Thr54-allele in FABP2 with its greater affinity for long-chain fatty acids, potentially results in an increased flux of fatty acids across the intestinal mucosa, allowing a higher absorption rate of fatty acids from ingested food and therefore higher plasma lipid levels, greater fat oxidation rates and decreased insulin action (Hegele 1998, De Luis *et al*. 2007). This may support a role for the *FABP2* Ala54Thr polymorphism in the etiology of metabolic disorders with Thr54-allele carriers potentially exhibiting higher BMIs, cholesterol, LDL, TG, apoB levels and insulin resistance.

2.3.5 Genotype and allele frequencies

The frequency of the wild-type *FABP2* Ala54Ala homozygous genotype is mostly between 50 and 57% in Caucasian populations with European ancestry (Table 2.5). However, a higher frequency is reported for African-American populations (60 to 63%) and American Indian tribes (>72%), while a lower frequency is found in populations from Asian origin such as the Japanese (mostly <50%). Although the frequency of the mutant Thr54Thr homozygous genotype is less than 10% in most Caucasian and African populations, association studies combine them with the Ala54Thr heterozygous genotype to make comparisons between all Thr54-allele carriers (Thr54Thr and Ala54Thr referred to as Thr54-allele carriers onwards) and the wild-type Ala54Ala genotype.

The highest frequency of the mutant Thr54 allele is found in the Japanese populations in the range of 34 to 40%, while the frequency in the Caucasian and African populations range between 24 to 30% and 19 to 23% respectively.

Table 2.5: Genotype frequencies of the *FABP2* Ala54Thr polymorphism and frequency of the mutant Thr54-allele.

Population	Weight or disease status	n	Ala/ Ala %	Ala/ Thr %	Thr/ Thr %	Thr54 allele %	References
Caucasian							
USA		714	51	41	8	29	Damcott *et al*. 2003
USA ♀		120	57	38	5	24	Lara-Castro *et al*. 2005
USA		71	48	45	7	30	Chiu *et al*. 2001a
USA		55	44	49	7	32	Chiu *et al*. 2001b
USA	DM2	287	52	37	11		Georgopoulos *et al*. 2000
USA (ethnicity not indicated)		122	48	48	4	28	Weiss *et al*. 2007
Chile: premenopausal ♀	All	63	37	47	16	40	Albala *et al*. 2004
	Obese	33	24	58	18		
	Non-obese	30	50	37	13		
Chile: aged 65-79 years		223	31	49	20	45	Albala *et al*. 2007
French Canadian ♂		217	46	47	7	31	Berthier *et al*. 2001
French Canadian children & adolescents		1742	55	38	7	26	Stan *et al*. 2005
Framingham ♂		907	53			47	Galluzzi *et al*. 2001
offspring study: ♀		1023	53			47	
European ♂ students		666	52	41	7	27	Tahvanainen *et al*. 2000
Sweden:	DM2	399	55	40	6	25	Carlsson *et al*. 2000
	Obese	59	53	39	8	28	
	Non-obese	59	52	36	12	30	
Greece		430	51	41	8	29	Tavridou *et al*. 2009
Germany: ♂ 45-65 years		700	51	41	8		Helwig *et al*. 2007
Finland	obese	170	50	45	5	29	Sipiläinen *et al*. 1997
Spain		538	52	40	8	27	Morcillo *et al*. 2007
Hispanic American							
Argentina		202	52	41	7	28	Gomez *et al*. 2007
Brazil						25	Canani *et al*. 2005
Mexican	obese	114	39	55	6	26	Martínez-López *et al*. 2007
African							
African-American ♀		103	63	36	0	19	Lara-Castro *et al*. 2005
African-American		1748	60	35	5	23	Lei *et al*. 1999
Native American							
Pima Indians	non-DM2	457	48	45	7	29	Baier *et al*. 1995
Aboriginal Chilean : Aymara (traditional lifestyle).		96	66	31	3	18	Pérez-Bravo *et al*. 2006
Aboriginal Chilean: Mapuche (urban)		111	46	44	10	31	Pérez-Bravo *et al*. 2006
Aboriginal Canadians		507	72	27	1	14	Hegele *et al*. 1996
Oji-Cree Canada adults			72	26	2	15	Pollex *et al*. 2006
Oji-Cree Canada adolescents			74	24	2	14	Pollex *et al*. 2006
Tonga		1022	76	23	1	12	Duarte *et al*. 2003
Asian							
Japanese ♂		395	43	45	12	34	Yamada *et al*. 1997
Japanese ♂ aged 40-65y:	Hyperglycemic	122	41	43	16	37	Ishii *et al*. 2001
	Normoglycemic	186	33	54	13	40	
	Hyperlipidemic	27	41	48	11	35	
	Normolipidemic	204	29	55	16	43	
Japanese ♂ aged 21-39y:		196	42	40	18		Ishii *et al*. 2001
Japanese ♀:	Obese (BMI>25)	80	41	40	19	39	Takakura *et al*. 2005
	Normal (BMI<25)	146	44	47	9	33	
Japanese		258	45	41	14	34	Hayakawa *et al*. 1999
Japanese-Americans		249	39	44	17		Nakanishi *et al*. 2004
Korean ♂ students		96	45	43	12	34	Kim *et al*. 2001
Indians		567				57	Leprêtre *et al*. 1998
Indians	DM2	773	48	42	10	30	Vimaleswaran *et al*. 2006
	NGT	899	51	42	7	28	

USA = United States of America, DM2 = Type 2 diabetes, NGT = Normal glucose tolerance

2.3.6 Association with obesity related phenotypes

Weight, BMI and obesity

The majority of studies summarized in Table 2.6 (25 out of 30 published studies) did not show an association between BMI and the *FABP2* Ala54Thr polymorphism. The five studies indicating a positive association between the *FABP2* Ala54Thr polymorphism and BMI all found a higher BMI in Thr54-allele carriers compared to the Ala54Ala homozygous genotype. These positive associations with BMI were reported in German women (Fisher *et al*. 2006), Mexican obese women (Martínez-López *et al*. 2007), non-obese Japanese-American women (Nakanishi *et al*. 2004) and Canadian Oji-Cree Indians (Hegele *et al*. 1996). In Japanese women, the retrospectively reported weight and BMI when they were 20 years old was higher for Thr54-allele carriers compared to Ala54Ala homozygotes (Takakura *et al*. 2005).

Only a few studies have compared the Ala54Thr allele frequency between obese and non-obese subjects. In Caucasians, two of the four published reports found an association between the Thr54-allele and obesity in Spaniards (Morcillo *et al*. 2007) and Greeks (Tavridou *et al*. 2009). No association with obesity was found in the other two studies conducted on Swedish (Carlsson *et al*. 2000) and Finnish (Sipiläinen *et al*. 1997) subjects. In Hispanic Americans, a case-control study reported a higher Thr54-allele frequency in obese compared to non-obese Chileans (Albala *et al*. 2004), while a cross-sectional study in Chileans (Albala *et al*. 2007) and a study in Mexicans found no association with obesity. In other non-Caucasian populations no associations between the Thr54-allele and obesity in African-Americans (Lei *et al*. 1999), Tongans (Duarte *et al*. 2003), Japanese (Nakanishi *et al*. 2004, Takakura *et al*. 2005), Asian Indians (Vimaleswaran *et al*. 2006) or Aboriginal Chileans with Asian and Native American ancestry (Pérez-Bravo *et al*. 2006) have been found.

In summary, the majority of studies reported no association between the *FABP2* Ala54Thr polymorphism and BMI or obesity. However, eight studies did report an association between Thr54-allele carriers or the Thr54-allele and a higher BMI or obesity prevalence in Caucasian, Hispanic American, Native American or Asian subjects.

Table 2.6: The association between the *FABP2* Ala54Thr polymorphism and weight, BMI and obesity.

Population group	Weight or diseases status	n	Design	Association with obesity		Association with BMI		Reference
				Yes	No	Yes	No	
Caucasian								
German ♂		700	Cross-sectional	ni	ni	-	✓	Helwig *et al*. 2007
Germany:	DM2	192	Case-control	ni	ni	T54 = ↑ BMI in ♀	-	Fisher *et al*. 2006
	Control	384						
USA		714	Cross-sectional	ni	ni	-	✓	Damcott *et al*. 2003
USA		71	Cross-sectional	ni	ni	-	✓	Chiu *et al*. 2001a
USA		55	Cross-sectional	ni	ni	-	✓	Chiu *et al*. 2001b
USA:	DM2	287		ni	ni	-	✓	Georgopoulos *et al*. 2000
French Canadian ♂		217	Volunteers in City	ni	ni	-	✓	Berthier *et al*. 2001
American ♀		120	Cross-sectional	ni	ni	-	✓	Lara-Castro *et al*. 2005
USA (ethnicity unknown)		122		ni	ni	-	✓	Weiss *et al*. 2007
Framingham offspring study		1930	Cross-sectional	ni	ni	-	✓	Galluzzi *et al*. 2001
European university ♂ students:	Cases (fathers had premature MI)	330	Case-control BMI compared in total sample	ni	ni	-	✓	Tahvanainen *et al*. 2000
	Controls	336						
Spain		538	Cross-sectional	Obese = ↑ T54	-	ni	ni	Morcillo *et al*. 2007
Greek		430	242 with DM2 vs 188 controls & 258 non-obese vs 172 obese.	Obese = ↑ T54	-	ni	ni	Tavridou *et al*. 2009
Sweden:	DM2 siblings	399		ni	ni	-	✓	Carlsson *et al*. 2000
	IGT siblings	93					(in siblings)	
	Normal BG siblings	195						
	Obese	59			✓			
	Non-obese	59						
Finland:	CHD	414	Cross sectional	ni	ni	-	✓	Erkkilä *et al*. 2002
Finland:	Obese	170	Case-control		✓	-	✓	Sipiläinen *et al*. 1997
	Non-obese	82						
Hispanic American								
Chilean ♀:	Obese	33	Case-control	Obese = ↑ T54	-	-	✓	Albala *et al*. 2004
	Non-obese	30						
Chili		223	Cross sectional	-	✓	-	✓	Albala *et al*. 2007
Argentina		202	Volunteers	ni	ni	-	✓	Gomez *et al*. 2007
Mexican	Obese	114		-	✓	T54 = ↑ BMI	-	Martínez-López *et al*. 2007

Table 2.6: *continue*

Population group	Weight or diseases status	n	Design	Association with obesity Yes	Association with obesity No	Association with BMI Yes	Association with BMI No	Reference
African								
African-American ♀		103	Cross-sectional	ni	ni	-	✓	Lara-Castro *et al*. 2005
African American:	Controls Diabetes Severe obesity Hyperinsulinemia	992 321 260 258	Case-control	-	✓	-	✓ (BMI) BMI at 25y ΔBMI since age 25 years	Lei *et al*. 1999
Native American								
Pima-Indians	no BMI reported	137	Subset of population sample to exclude related subjects	ni	ni	-	not with weight	Baier *et al*. 1995
Aboriginal Chileans: Aymara (rural) Mapuche (urban)		 96 111		-	✓	-	✓	Pérez-Bravo *et al*. 2006
Aboriginal Canadians		507	All subjects in community	ni	ni	T54A = ↑ BMI (T54T excluded from analysis)	-	Hegele *et al*. 1996
Tonga		1022	Random population sample	-	✓	-	✓	Duarte *et al*. 2003
Asian								
Japanese ♂		395	Cross-sectional	ni	ni	-	✓	Yamada *et al*. 1997
Japanese ♂:age=21-39 age=40-65		196 186		ni	ni	- -	✓ ✓	Ishii *et al*. 2001
Japanese ♀:	Obese (BMI>25) Non-obese (BMI<25)	80 146	Case-control	-	✓	T54 = ↑ BMI at age 20 years	✓	Takakura *et al*. 2005
Japanese		285		ni	ni	-	✓	Hayakawa *et al*. 1999
Japanese-Americans		249	Follow-up of 7.8 years	-	✓	T54 non-obese ♀ = ↑ BMI	in ♂ & the obese ♀	Nakanishi *et al*. 2004
Japanese school children	Morbidly obese Non-obese	370 463	Case-control	-	✓	ni	ni	Endo *et al*. 2001
Korean ♂		96		ni	ni	-	✓	Kim *et al*. 2001
Pondicherian Tamil Indians from South India:	49 families with at least 2 sibships had DM2.	567	Purposively selected sample, Compared 49 DM2 vs 50 controls for BMI	ni	ni	-	✓	Leprêtre *et al*. 1998
Asian Indians		773 899	Case-control for DM2 vs. NGT compared obese vs. non-obese	-	✓	ni	ni	Vimaleswaran *et al*. 2006

ni = not investigated, BMI = body mass index, DM2 = Type 2 diabetes mellitus, ♀ = female, ♂ = male, CHD = coronary heart disease, NGT = normal glucose tolerance, IGT = impaired glucose tolerance, MI = myocardial infarction, BG = blood glucose.

Measures of body fat distribution and content

Only a limited number of published studies included variables measuring body fat distribution and content in research focused on the *FABP2* Ala54Thr polymorphism (Table 2.7). Martínez-López *et al*. (2007) indicated that Mexican Thr54-allele carriers had a higher waist circumference (WC) compared to Ala54Ala homozygotes. However, all prior studies failed to find any association with either WC or waist-hip ratio in Caucasians (Sipiläinen *et al*. 1997, Carlsson *et al*. 2000, Tahvanainen *et al*. 2000, Berthier *et al*. 2001, Chiu *et al*. 2001a, Chiu *et al*. 2001b, Erkkilä *et al*. 2002, Helwig *et al*. 2007), Hispanic Americans (Albala *et al*. 2004, Albala *et al*. 2007), Native Americans (Duarte *et al*. 2003, Pérez-Bravo *et al*. 2006) or Asians (Leprêtre *et al*. 1998, Hayakawa *et al*. 1999, Kim *et al*. 2001).

When considering body fat content Hegele *et al*. (1996) found that Thr54-allele carriers among Canadian Oji-Cree Indians had a higher percentage body fat while Thr54Thr homozygotes among Japanese men had higher levels of visceral adipose tissue as indicated by a higher intra-abdominal fat thickness and intra-abdominal fat/ subcutaneous fat ratio (Yamada *et al*. 1997). However, no association between the *FABP2* Ala54Thr polymorphism and percentage body fat was found in Japanese women (Takakura *et al*. 2005), Pima-Indians (Baier *et al*. 1995) or Tongans (Duarte *et al*. 2003). In contrast with this, Caucasian premenopausal women who were Thr54-allele carriers had lower levels of total abdominal adipose tissue and abdominal subcutaneous adipose tissue compared with Ala54Ala homozygotes (Lara-Castro *et al*. 2005). This association was not found for African-American women (Lara-Castro *et al*. 2005). In other populations from European ancestry, no associations were found with percentage body fat (Sipiläinen *et al*. 1997, Martínez-López *et al*. 2007, Weiss *et al*. 2007), fat mass (Damcott *et al*. 2003, Lara-Castro *et al*. 2005), lean mass (Lara-Castro *et al*. 2005) or visceral adipose tissue (Berthier *et al*. 2001, Lara-Castro *et al*. 2005).

In summary, in only four of the 23 published studies positive associations were reported. In three of these studies the mutant Thr54-allele was associated with measures indicative of a higher body fat content. In contrast, one study associated the Thr54-allele with a lower total and subcutaneous abdominal tissue. The bulk of evidence at this point in time points to no association between the *FABP2* Ala54Thr polymorphism and both body fat distribution and content.

Table 2.7: The association between the *FABP2* Ala54Thr polymorphism and measures of body fat distribution and content

Population	Associations	No associations	Reference
Caucasian			
German ♂	-	WC	Helwig *et al*. 2007
USA	-	Fat mass	Damcott *et al*. 2003
USA	-	WHR	Chiu *et al*. 2001a
USA	-	WHR	Chiu *et al*. 2001b
French Canadian ♂	-	WC, VAT area	Berthier *et al*. 2001
USA	T54 = ↓ TAT, SAAT	Fat mass, lean mass, VAT	Lara-Castro *et al*. 2005
USA	-	% body fat	Weiss *et al*. 2007
European ♂ students	-	WHR	Tahvanainen *et al*. 2000
Sweden	-	WHR	Carlsson *et al*. 2000
Finnish obese subjects	-	WC, WHR, body fat %, LBM	Sipiläinen *et al*. 1997
Finnish subjects with CHD	-	WC, WHR	Erkkilä *et al*. 2002
Hispanic American			
Chilean ♀	-	WC, WHR	Albala *et al*. 2004
Chili	-	WC	Albala *et al*. 2007
Mexico	T54 = ↑ WC	body fat %	Martínez-López *et al*. 2007
African			
African-American	-	Fat mass, lean mass, TAT, VAT, SAAT	Lara-Castro *et al*. 2005
Native Americans			
Pima-Indians	-	body fat %	Baier *et al*. 1995
Tonga	-	WHR, FFM, % fat	Duarte *et al*. 2003
Aboriginal Chileans	-	WHR, WC	Pérez-Bravo *et al*. 2006
Aboriginal Canadian	T54A = ↑ body fat % (T54T were excluded)		Hegele *et al*. 1996
Asian ancestry			
Japanese ♂	T54T = ↑ AFT, ASR	SFT	Yamada *et al*. 1997
Japanese obese ♀	-	% body fat	Takakura *et al*. 2005
Korean ♂	-	WHR	Kim *et al*. 2001
Japanese	-	WHR	Hayakawa *et al*. 1999
Indians	-	WHR	Leprêtre *et al*. 1998

T54 = Thr54-allele carriers, T54T = Thr54 homozygotes, T54A = heterozygotes, WC = waist circumference, WHR = waist-hip-ratio, VAT = visceral abdominal tissue, TAT = total abdominal adipose tissue, SAAT = subcutaneous abdominal fat, LBM = lean body mass, FFM = fat free mass, AFT = intra-abdominal fat thickness, SFT = Subcutaneous fat thickness, ASR = Intra-abdominal fat/subcutaneous fat ratio.

2.3.7 Associations with health indicators

Indicators of glucose and insulin homeostasis

Several studies have investigated the association between the *FABP2* Ala54Thr polymorphism and fasting glucose, insulin and post-prandial glucose and insulin levels (Table 2.8). The Thr54-allele has been found to be associated with higher fasting glucose levels in Caucasian (Chiu *et al*. 2001b, Weiss *et al*. 2007) and male Japanese subjects (Ishii *et al*. 2001). Higher fasting insulin levels were found in Thr54-allele carriers from Caucasian (Weiss *et al*. 2007), Hispanic-American (Albala *et al*. 2004), Pima-Indian (Baier *et al*. 1995) and Asian populations (Yamada *et al*. 1997, Kim *et al*. 2001). However, in the majority of studies across all populations no association with fasting glucose or insulin was found.

When challenged with a 75g oral glucose tolerance test, Thr54-allele carriers responded with higher post-prandial glucose levels in Caucasian (Chiu *et al*. 2001a) and Asian populations (Vimaleswaran *et al*. 2006) and higher post-prandial insulin levels in Caucasians (Chiu *et al*. 2001a, Helwig *et al*. 2007), Pima-Indians (Baier *et al*. 1995) and Asians (Yamada *et al*. 1997, Vimaleswaran *et al*. 2006). Furthermore, Weiss *et al*. (2007) indicated that fewer Thr54-allele carriers had a normal glucose tolerance compared to Ala54Ala homozygotes in Caucasians.

Thr54-allele carriers were reported to have a higher prevalence of MetS in Aboriginal Canadian adolescent girls (Pollex *et al*. 2006) and Asians (Vimaleswaran *et al*. 2006). These genotypes were also associated with insulin resistance as assessed by the homeostasis model (HOMA-IR) in Caucasians (Helwig *et al*. 2007) and Japanese (Yamada *et al*. 1997). Decreased insulin sensitivity and a decreased insulin sensitivity index (ISI) were also reported in Thr54-allele carriers in Caucasian populations (Chiu *et al*. 2001a, Chui *et al*. 2001b, Weiss *et al*. 2007). Similarly, in Pima Indians the insulin-stimulated glucose uptake rate was lower in Thr54-allele carriers (Baier *et al*. 1995). The Thr54Thr homozygous genotype was also associated with an increased prevalence and incidence of type-2 diabetes over a four year period in Chileans (Albala *et al*. 2007). However, several studies did not find an association between the *FABP2* Ala54Thr polymorphism and HOMA-IR in Caucasians (Damcott *et al*. 2003, De Koning *et al*. 2008), Hispanic Americans (Albala *et al*. 2004, Albala *et al*. 2007, Martínez-López *et al*. 2007) and Asians (Hayakawa *et al*. 1999, Ishii *et al*. 2001, Nakanishi *et al*. 2004, Takakura *et al*. 2005, Vimaleswaran *et al*. 2006, De Koning *et al*. 2008) or with type-2 diabetes in Caucasians (Tavridou *et al*. 2009), Native Americans (Baier *et al*. 1995, Hegele *et al*. 1996), African-Americans (Lei *et al*. 1999) and Asians (Vimaleswaran *et al*. 2006). Two studies indicated increased $HbA1_C$ levels in Asian Thr54-allele carriers with a normal glucose tolerance (Vimaleswaran *et al*. 2006) and obese Asians (Takakura *et al*. 2005) while this association was not found in Asian type-2 diabetics (Vimaleswaran *et al*. 2006), Tongans (Duarte *et al*. 2003) or other Asians (Leprêtre *et al*. 1998).

In summary, across all ancestral groups the positive association studies all indicated that the Thr54-allele or Thr54-allele carriers were associated with higher fasting glucose and insulin levels, higher post-prandial insulin levels, decrease insulin sensitivity and insulin stimulated glucose uptake, increased insulin resistance, a higher $HbA1_C$ and a higher prevalence of MetS and type-2 diabetes.

Blood lipid profile and blood pressure

Thr54-allele carriers in Caucasian (Carlsson *et al*. 2000, Berthier *et al*. 2001), Hispanic American (Martinez-Lopez *et al*. 2007), Native American (Hegele *et al*. 1996) and Asian (Nakanishi *et al*. 2004, Vimaleswaran *et al*. 2006, De Koning *et al*. 2008) populations have been found to have higher fasting TG levels (Table 2.8). In a population of persons with type 2 diabetes, fasting TG and postprandial TG after fat ingestion increased linearly only in homozygotes for the Thr54-allele (Georgopoulos *et al*. 2000).

Thr54-allele carriers in Caucasian (Carlsson *et al*. 2000, Galluzzi *et al*. 2001, Stan *et al*. 2005), Hispanic-American (Martínez-López *et al*. 2007) and Asian (Nakanishi *et al*. 2004, Vimaleswaran *et al*. 2006) populations have also

been found to have higher TC, LDL and VLDL levels and lower HDL levels. However, in Tongans the Thr54-allele carriers were found to have lower TC and LDL levels (Duarte *et al*. 2003). Some studies have also found associations between Thr54-allele carriers and other indicators of lipid metabolism, including higher levels of ApoB (Stan *et al*. 2005, Galluzzi *et al*. 2001), TNFα (Albala *et al*. 2004), leptin (Albala *et al*. 2004) and fasting fat oxidation rate (Baier *et al*. 1995, Kim *et al*. 2001). However, many studies found no associations between the *FABP2* Ala54Thr polymorphism and TC, TG, LDL, HDL, LDL/HDL ratio, TC/HDL ratio, FFA, ApoAI and ApoB.

From the above it may be deduced that Thr54-allele carriers might have an increased risk to develop CHD. Research by Carlsson *et al*. (2000) supports this possibility as they found that the Thr54-allele carriers had a higher stroke prevalence and higher prevalence of parents with a stroke history (Carlsson *et al*. 2000). The Thr54-allele carriers were also associated with a higher MI prevalence in subjects with MetS (Oguri *et al*. 2009) and a higher prevalence of transient ischemic attacks and non-cardioembolic infarction at a younger age (Wanby *et al*. 2004). However, Galluzzi *et al*. (2001) and Gomez *et al*. (2007) found no association between the *FABP2* Ala54Thr polymorphism and CHD or cardiovascular risk. It is also clear from the studies summarized in Table 2.9 that the *FABP2* Ala54Thr polymorphism does not seem to influence blood pressure (Yamada *et al*. 1997, Leprêtre *et al*. 1998, Hayakawa *et al*. 1999, Lei *et al*. 1999, Carlsson *et al*. 2000, Chiu *et al*. 2001a, Chiu *et al*. 2001b, Ishii *et al*. 2001, Kim *et al*. 2001, Nakanishi *et al*. 2004, Albala *et al*. 2007, Gomez *et al*. 2007).

In summary, although many studies did not find an association between the *FABP2* Ala54Thr polymorphism and lipid profile and heart disease risk the studies summarized in Table 2.8 point to the possibility that having a Thr54-allele may predispose an individual to an unfavourable lipid profile and a higher heart disease risk.

Table 2.8: The association between the *FABP2* Ala54Thr polymorphism and indicators of glucose and insulin homeostasis, blood lipid profile and blood pressure.

Population	Glucose and insulin homeostasis		Blood lipids and blood pressure (BP)		Reference
	Association	No association	Association	No associations	
Caucasian populations					
German ♂	-	Fasting & postprandial insulin, glucose, HOMA after an OGTT	-	TC, LDL/HDL ratio	Helwig *et al*. 2007
German	T54T = ↓ risk of T2DM, ↓ $HbA1_c$ in ♀	-	ni	ni	Fisher *et al*. 2006
USA	-	HOMA-IR	-	TC, TG, FFA	Damcott *et al*. 2003
USA	T54 = ↑ glucose & insulin 2h after OGTT, ↓ %S, ISI_s	fasting glucose, insulin, β cell function	-	BP	Chiu *et al*. 2001a
USA	T54 = ↑ fasting glucose, ↓ %S	fasting insulin, β cell function	-	TC, TG, LDL, HDL, BP	Chiu *et al*. 2001b
USA	-	$HbA1_c$	T54 = ↑ TC, TG, non-HDL cholesterol	HDL, LDL	Georgopoulos *et al*. 2000
America (ancestry not indicated)	Normal OGTT = ↓ T54 T54 = ↓ ISI, ↑ fasting glucose, insulin AUC, fasting insulin	2h insulin, 2h glucose, glucose AUC	-	fasting TG, fasting FA, fasting & 2h lipid oxidation	Weiss *et al*. 2007
French Canadian ♂	-	Glucose, insulin	-	TC, LDL, VLDL, HDL, TG, FFA, ApoA1, ApoB	Berthier *et al*. 2001
French Canadian youth	-	IRS	high TG + T54T = ↑ TC, LDL, apoB	-	Stan *et al*. 2005
French Canadian youth without IRS	-	Glucose, insulin	-	TC, LDL, HDL, TG FFA, ApoB, ApoAI	Stan *et al*. 2005
French Canadian youth with IRS	-	Glucose, insulin	T54 = ↑ TC, LDL	HDL, TG FFA, ApoB, ApoAI	Stan *et al*. 2005
Framingham offspring study	-	-	T54 ♂ = ↑ LDL, ApoB, small VLDL, large HDL T54 ♀ = ↑ TC, LDL	♂ & ♀ = HDL, TG, ApoAI, CHD. In ♂ = TC ♀ = ApoB, all lipoprotein subclass profiles.	Galluzzi *et al*. 2001
Greek	-	DM2	ni	ni	Tavridou *et al*. 2009
European ♂ university students	-	fasting glucose, insulin, & glucose & insulin changes after OGTT	-	TC, TG, HDL, LDL, ApoB, ApoAI	Tahvanainen *et al*. 2000

Table 2.8: *continue*

Population	Glucose and insulin homeostasis		Blood lipids and blood pressure (BP)		Reference
	Association	No association	Association	No associations	
Caucasian populations					
Sweden	-	2-h glucose, insulin, DM	T54 siblings = ↑ TG, TC T54 offspring = ↑ parental stroke Hx. Stroke Hx =↑ T54	fasting NEFA, BP	Carlsson *et al*. 2000
Finnish obese subjects	-	glucose, insulin, fasting glucose oxidation.	-	TC, HDL, LDL, VLDL, TG, FFA, BMR, fasting lipid oxidation	Sipiläinen *et al*. 1997
Finnish with CHD	-	MetS, glucose	-	TC, TG, CHD, BP	Erkkilä *et al*. 2002
Canada	-	HOMA-IR	-	TG	De Koning *et al*. 2008
Hispanic American					
Chilean ♀	T54T = ↑ insulin	HOMA-IR	T54T = ↑ TNFα, Leptin	TG	Albala *et al*. 2004
Chili	T54T = ↑ DM2 prevalence, ↑ DM2 incidence over 4 yrs	Glucose, insulin, HOMA-IR	-	TC, TG, HDL, BP	Albala *et al*. 2007
Argentina	-	Glucose	-	TC, BP, Cardiovascular risk	Gomez *et al*. 2007
Mexico	-	Glucose, insulin, HOMA-IR	T54 = ↑ TC, LDL, VLDL, TG	HDL, total lipids, ApoAI, ApoB	Martínez-López *et al*. 2007
Native American					
Pima-Indians (non-diabetics)	T54 = ↑ insulin, ↓ insulin-stimulated glucose uptake, ↑ insulin after 75g OGTT.	glucose, DM2	T54 = ↑ fasting fat oxidation rate	-	Baier *et al*. 1995
Aboriginal Chileans	-	HOMA-IR, fasting insulin	-	TG	Pérez-Bravo *et al*. 2006
Aboriginal Canadian	-	DM2, impared glucose tolerance, insulin	T54A = ↑ TG (Thr54Thr excluded from analysis)	TC, HDL, LDL	Hegele *et al*. 1996
Tonga ♂	A54A = ↑ 1h glucose	fasting BG, 2h glucose, insulin, $HbA1_c$	T54 = ↓ TC, LDL	HDL, TG, total/HDL ratio	Duarte *et al*. 2003
Tonga ♀	-	fasting BG, 1h glucose, 2h glucose, insulin, $HbA1_c$	T54 = ↓ TC, HDL, LDL	TG, total/HDL ratio	Duarte *et al*. 2003
Oji-Cree Canada	T54 = ↑ MetS prevalence in adolescent ♀	MetS in adults	ni	ni	Pollex *et al*. 2006

Table 2.8: *continue*

Population	Glucose and insulin homeostasis		Blood lipids and blood pressure (BP)		Reference
	Association	No association	Association	No associations	
African					
African-American	-	glucose, insulin, DM2	-	HDL, LDL, TG, BP, total kJ intake, leisure index	Lei *et al*. 1999
Asian ethnicity					
Japanese ♂	T54T = ↑ insulin, ↑ HOMA-IR, ↑ insulin levels 120min after 75g oral glucose load.	Fasting glucose, 75g Glucose tolerance test	-	BP, TC, HDL, TG	Yamada *et al*. 1997
Japanese ♂	T54 normoglycemic ♂ = ↑ glucose	Insulin, HOMA-IR	-	TC, TG, HDL, LDL, FFA, Leptin, heart rate, BP	Ishii *et al*. 2001
Japanese obese ♀	T54 = ↑ HbA1c	Fasting glucose, insulin, HOMA-IR	-	TC, TG, LDL, free T_3, free T_4, TSH	Takakura *et al*. 2005
Japanese	-	Fasting glucose, insulin, HOMA-IR	-	TC, TG, HDL, FFA, BP, uric acid	Hayakawa *et al*. 1999
Japanese-American obese ♀	-	Glucose, HOMA-IR	T54 = ↑ TC, LDL	TG, HDL, BP	Nakanishi *et al*. 2004
Japanese-American obese ♂	-	Glucose, HOMA-IR	-	TC, TG, LDL, HDL, BP	Nakanishi *et al*. 2004
Japanese-American non-obese	-	Glucose, HOMA-IR	T54 = ↑ LDL in ♀ T54 = ↑ TG in ♂	TC, HDL, BP, TG in ♀ & LDL in ♂	Nakanishi *et al*. 2004
Japanese obese children	-	-	T54T = ↓ p-AA, D6D (ω6 PUFA pathway)	-	Okada *et al*. 2006
Korean ♂	T54 = ↑ insulin	Fasting glucose, 2h glucose, 2h insulin	T54 = ↑ fat oxidation	TC, TG, HDL, LDL, FFA, BP, leptin, carbohydrate oxidation, BEE, BMR	Kim *et al*. 2001
Chinese Canadians	-	HOMA-IR	-	TG	De Koning *et al*. 2008
South-Asian Canadians	-	HOMA-IR	Thr54 = ↑ TG	-	De Koning *et al*. 2008
Asian Indians	NGT T54= ↑ 2h glucose, $HbA1_c$, 2h insulin, MetS = ↑ T54	DM2. In DM2 & NGT: glucose, HOMA-IR, insulin In DM2: 2h glucose, 2h insulin, $HbA1_c$	NGT T54 = ↑ TG, LDL, ↓ HDL DM2 T54 = ↑ TG ↑ TG = ↑ T54 vs low TG	In NGT: TC In DM2: TC, HDL, TG, LDL	Vimaleswaran *et al*. 2006
Pondicherian Tamil Indians	-	$HbA1_c$	-	TC, TG, BP	Leprêtre *et al*. 1998

Ni = not investigated, MetS = Metabolic Syndrome, DM2 = Type 2 Diabetes Mellitus, TG = triglycerides, TC = Total Cholesterol, HOMA-IR = Homeostasis model assessment of insulin resistance, HDL = High density lipoprotein, VLDL = Very low-density lipoprotein, LDL = Low density lipoprotein, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, FFA = free fatty acids, CHD = Coronary heart disease, Lipoprotein subclass profiles includes small, intermediate & large VLDL, HDL, LDL, OGTT = oral glucose tolerance test, OMTT = oral metabolic tolerance test, PPLT = postprandial lipemia test, OFFT = oral fat tolerance test, AUC = area under curve, ISI = insulin sensitivity index, %S = insulin sensitivity, p-AA = plasma arachidonic acid, D6D = delta-6 desaturase.

Associations with post prandial lipid levels

To date nine studies examined the role of the *FABP2* Ala54Thr polymorphism on post-prandial responses to oral fat tolerance tests (OFTT) (Table 2.9). As no universally standard OFTT is available, different OFTT protocols were used in these studies. In general subjects fasted overnight and received the OFTT (with different compositions as specified in Table 2.9) either in the form of a drink or meal in the morning. Blood samples were collected before and every half hour or hour or two hours for a period of six to eight hours to measure post-prandial changes.

Four studies showed that Thr54Thr homozygotes had elevated post-prandial levels of chylomicron cholesterol (Ågren *et al*. 1998), total TG (Ågren *et al*. 1998, Georgopoulos *et al*. 2000, Helwig *et al*. 2007), HDL-TG (Berthier *et al*. 2001), chylomicron TG (Ågren *et al*. 1998, Georgopoulos *et al*. 2000), VLDL-TG (Ågren *et al*. 1998), LDL/HDL ratio, TG increase over five hours (Helwig *et al*. 2007) and elevated levels of long-chain fatty acids such as stearic and palmitic acids in chylomicrons and VLDLs (Ågren *et al*. 2001). However, four studies reported no effect on post-prandial TG levels (Pratley *et al*. 2000, Tahvanainen *et al*. 2000, Dworatzek *et al*. 2004, Weiss *et al*. 2007). Weiss *et al*. (2007) also reported that an OFTT caused increased lipid oxidation rates in Thr54-allele carriers. This was supported by one study showing higher levels of FFA (Pratley *et al*. 2000) in Thr54Thr homozygotes following an OFTT, however four studies did not find this association with FFA levels (Ågren *et al*. 1998, Berthier *et al*. 2001, Dworatzek *et al*. 2004, Weiss *et al*. 2007). It was further found that a OFTT consisting of 85% fat of TE (Pratley *et al*. 2000) or mixed meals OFTTs consisting of 40% fat and 40% carbohydrates (Baier *et al*. 1995) or 51.6% fat and 29.6% carbohydrates (Helwig *et al*. 2007) might be harmful for Thr54-allele carriers as it decreases insulin sensitivity. Although not significant, Pratley *et al*. (2000) showed a tendency to higher post-prandial NEFA and insulin levels after the ingestion of a mixed meal (40% fat and 40% carbohydrates) in Thr54-allele carriers. These differences in outcomes might be explained by the different OFTT protocols used. However, factors such as differences in sample size, ethnicity, age and body fatness of populations investigated as well as differences in accounting for confounding factors such as habitual physical activity levels and dietary composition also need to be borne in mind (Morcillo *et al*. 2007, Weiss *et al*. 2007).

Five studies also examined the association between the *FABP2* Ala54Thr polymorphism and the ingestion of specific fats on insulin resistance or post-prandial changes (Table 2.10). It was found that replacing half of the C18:1 cis fatty acids in a meal with C18:1 trans fatty acids resulted in greater post-prandial lipogenesis (measured with increased TG fractional synthetic rate) and a two-fold greater post-prandial glucose response in Thr54-allele carriers (Lefevre *et al*. 2005). Thr54-allele carriers may respond more negatively to a high SFA diet than non-carriers as a diet containing 38% fat of TE (20% SFA) caused higher FFA levels and lower peripheral insulin sensitivity in carriers. These subjects may respond more positively to a low fat (<30% fat of TE) high carbohydrate (57% of TE) diet or a high MUFA (22% MUFA and 38% fat of TE) diet because these diets had no negative post-prandial effects in carriers (Marín *et al*. 2005). Similar beneficial effects of MUFA use in Thr54-allele carriers were illustrated by Morcillo *et al*. (2007). These researchers found that Thr54-allele carriers who use olive oil as the cooking oil in their homes were less insulin resistant while Thr54-allele carriers using

sunflower oil were the most insulin resistant according to higher HOMA-IR levels (Morcillo *et al*. 2007). A study in rats also showed that the Fabp2 protein had a greater affinity for linoleic acid (PUFA) than for oleic acid (MUFA) (Richieri *et al*. 1994). Thus, Thr54-allele carriers, who have a higher binding affinity for fat (Baier *et al*. 1995), may experience above-mentioned negative effects with the consumption of a high linoleic acid diet. A diet high in MUFA may protect Thr54-allele carriers against these effects as indicated by the mentioned studies and the fact that FABP2 does not have a high affinity for these fatty acids. However, contradictory results were found by Dworatzek *et al*. (2004) indicating that an olive oil OFTT was associated with higher chylomicron cholesterol in Thr54-allele carriers, but no negative effect was experienced after a safflower oil or butter OFTT. Another report also showed that replacing 20g triacylglycerol (TAG) with a 20g diacylglycerol (DAG) test oil may positively effect Thr54-allele carriers as indicated by lower VLDL-phospholipids and a lower visceral fat/total body fat percentage (VF/TF %) after four weeks of intervention (Yanagisawa *et al*. 2003).

Not included in Table 2.9 is another study that investigated the effect of the *FABP2* Ala54Thr polymorphism and dietary soluble and insoluble fibre on blood lipid levels. In a year long cross-over study with 43 subjects it was shown that Thr54-allele carriers benefit more from a high soluble fibre diet than an insoluble fibre diet to decrease plasma TC, LDL and apoB levels compared to Ala54Ala homozygous subjects (Hegele *et al.* 1997).

In summary these results suggest that Thr54-allele carriers may experience higher post-prandial lipid levels and decreased insulin sensitivity following an OGTT or diets high in PUFAs, SFAs or trans fatty acids. However, a high intake of MUFAs could counter these effects. It is known that chronic exposure to postprandial hyperlipidemia results in accumulation of intracellular fat in muscle and adipocytes which ultimately impairs insulin action, causing glucose intolerance, and increases the risk for the development of Type-2 diabetes (Baier *et al*. 1995, Weiss *et al*. 2002). Therefore, these results may indicate that Thr54-allele carriers could benefit from specific dietary fat manipulation strategies to decrease their overall risk for the development of CVD and Type-2 diabetes.

Table 2.9: The association between the *FABP2* Thr54Ala polymorphism and postprandial lipid and insulin parameters following OFTTs or interventions with different fatty acids.

Population	n	Genotype (n or %)	OFTT or dietary intervention with different fatty acids	Results: Postprandial associations	No associations	Reference
OFTT						
Finland	15	T/T=8 A/A=7	OFTT: meal = 0.75g/kg body weight. 7.6g rice cake + 10g cheese + 5 min later 100ml cream mixture + heptadecanoic acid + 20ml fish oil.	Thr54Thr: = ↑ AUC for TG, chylomicron TG, VLDL-TG, = ↑ LDL-TG from 6-8 hrs = ↑ chylomicron cholesterol = Correlation between TG & insulin response	insulin response FFA	Ågren *et al*. 1998
Finland	15	T/T=8 A/A=7	OFTT: meal = 52g fat/ 70kg body weight. 7.6g rice cake + 10g cheese + 5 min later 100ml cream mixture + heptadecanoic acid + 20ml fish oil.	Thr54Thr = ↑ increases in most post prandial 14 to 18 carbon chain FA in chylomicrons & VLDL	-	Ågren *et al*. 2001
Pima Indians	137	T/T=12 T/A=57 A/A=68	Mixed meal: 30% of weight maintenance kJ, 20% protein, 40% carbohydrate, 40% fat	Thr54 = ↑ postprandial insulin response	-	Baier *et al*. 1995
French Canadian ♀	20	T/A=10 A/A=10	OFTT: Meal = 60g lipid/l m^2 body surface area; 1800-2200kcal, 64% fat, 18% carbohydrate, 18% protein.	Ala54Thr = ↑ postprandial HDL-TG at 0 & 4h = ↑ AUC for HDL-TG = TG in large-TRL correlated with HDL-TG	BG, FFA, LDL, TRL, insulin	Berthier *et al*. 2001
Canadian subjects, ethnicity not specified	22	T54=11 A/A=11	Each subject underwent 3 OFTT. Test 1 = safflower oil, Test 2 = olive oil, Test 3 = butter. All prepared as milk shakes	Thr54 = ↓ insulin/C-peptide ratio, AUC for insulin = suggest greater hepatic insulin clearance = ↑ chylomicron cholesterol after olive oil	TC, TG, FFA, chylomicron, BG.	Dworatzek *et al*. 2004
Caucasian ♂ T2DM	15	T/T=6 A/A=9	OFTT: shake (egg white, sweet-and-low, fruit flavour, and 55g corn oil/ M^2 of body surface).	Thr54Thr = ↑ postprandial TG, ↑ AUC of TG levels in the chylomicron subfraction	VLDL-TG	Georgopoulos *et al*. 2000
German ♂	700	A/A=51% A/T=41%; T/T=8%	Liquid mixed meal = 4392kJ, 51.6% fat, 29.6% carbohydrates, 11.9% protein.	Thr54Thr = ↑ postprandial TG, ↑ AUC of TG, ↑ TG increase, ↑ LDL/HDL ratio, = ↑ AUC of postprandial insulin levels	-	Helwig *et al*. 2007
Pima Indians	18	T/T=9 A/A=9	Subjects received weight-maintaining diet for 14 days. Diet = individualized kJ, 40% fat, 40% carbohydrate, 20% protein After day 5: **Mixed meal test** = 35% of calculated 24h energy needs, 40% carbohydrates, 20% protein, 40% fat. **High fat meal test** = 1362 kcal, 85% fat.	Mixed meal: Thr54 tended to have ↑ postprandial NEFA and insulin levels. High fat meal: Thr54 = ↑ insulin at 1h, ↑ NEFA at 7h.	TG, BG, Insulin, NEFA TG, BG	Pratley *et al*. 2000

Table 2.9: *continued*

Population	n	Genotype (n or %)	OFTT or dietary intervention with different fatty acids	Results: Postprandial associations	No associations	Reference
OFTT						
European male students	666	A/A=52%; A/T=41%; T/T=7%	OFTT = liquid consisting of 42g SFA, 22g protein, 56g carbohydrate, 6186 kJ.	-	TG	Tahvanainen *et al*. 2000
USA	36	T/T=2 A/T=22 A/A=12	OFTT = meal with whipping cream, sugar, chocolate syrup and nonfat powdered milk. Size of meal = 386g/ $2m^2$ body surface area. 386g meal = 1362 kcal, 84% fat	Thr54 = ↑ lipid oxidation rates at 4h	TG, FA, 2h lipid oxidation	Weiss *et al*. 2007
Fatty acid interventions						
Caucasian: Spain	538	A/A=52%; A/T=40%; T/T=8%	No intervention. On home visits investigators took sample of cooking oil used. Samples analyzed & categorized in 3 groups: sunflower oil, olive oil & mixed oil.	Thr54 + sunflower oil = ↑ HOMA-IR Thr54 + olive oil = lowest HOMA-IR values	-	Morcillo *et al*. 2007
Caucasian: Spain ♀ only	59	A/A=47% A/T=46%; T/T=7%	All completed 3 diets, each lasting 28 days. All started with diet 1, then randomly assigned (cross-over design) to follow diet 2 and 3. All meals served in student cafeteria. All diets = 10200 kJ, 15% protein, 6% PUFA and: Diet 1: ↑ SFA diet=47% carb, 38% fat, 20% SFA, 12% MUFA Diet 2: ↓ fat = 57% carb, 28% fat, <10% SFA, 12% MUFA Diet 3: ↑ MUFA diet = 47% carb, 38% fat, <10% SFA, 22% MUFA	Thr54 = ↑SSPG (less peripheral insulin sensitivity) after SFA diet than MUFA diet, = ↑ FFA after consuming SFA diet (not seen after carb or MUFA diets).	-	Marín *et al*. 2005
USA (include 18% African Americans)	22	T54=10 A/A=12	Subjects received low fat diet for 16 days (24% fat, 7% SFA, 7% PUFA, 10% MUFA). Days 10 and 16, consumed 1 of 2 OFTT Both meals = egg, mushroom and pototo quiche = 40% of energy need, 50% total fat, 15% SFA, 15% PUFA and: C18:1*cis* test meal: 20% *cis*-MUFA C18:1*trans* test meal: 10% cis-MUFA +10% trans-MUFA	Postprandial, C18:1 trans resulted in: Thr54 = ↑ increments in glucose, = ↑ triacylglycerol fractional synthetic rate	TG, FFA, fat oxidation, carbohydrate oxidation.	Lefevre *et al*. 2005
♀ Japanese university students majoring in Nutritional science	49	A/A=17% A/T=56%; T/T=27%	9-day conditioning constant diet. Day 10: DAG or TAG for 8 weeks. Diets = identical except for either 20g DAG or TAG test oils/day.	Ala54Thr: TAG = ↑ VLDL-phospholipids (week 4-8) DAG = ↓ VLDL-phospholipids (week 4-8) = ↓ VF/TF %.	FFA, TC, VLDL-cholesterol, VLDL-TG	Yanagisawa *et al*. 2003

SSPG = steady state plasma glucose, SSPI = steady state plasma insulin, NEFA = non-esterified fatty acids, TAG = triacylglycerol, DAG = Diacylglycerol, VF/TF = Visceral fat/ total fat ratio, TRL = triglyceride rich lipoprotein, TG = triglycerides, TC = total cholesterol, VLDL = very low density lipoprotein, LDL = low density lipoprotein, HDL = high density lipoprotein, BG = blood glucose, AUC = area under the curve, OFTT = oral fat tolerance test, FFA = free fatty acids, SFA = saturated fatty acids, PUFA = poly-unsaturated fatty acids, MUFA = mono-unsaturated fatty acids, TE = total energy, HOMA-IR = Homeostasis model assessment of insulin resistance.

2.3.8 Associations with weight loss outcomes

The association between the *FABP2* Ala54Thr polymorphism and weight loss following a weight loss intervention has been investigated in Spaniards (De Luis *et al*. 2006, De Luis *et al*. 2008a, De Luis *et al*. 2008b) and Japanese (Takakura *et al*. 2005) (Table 2.10). In one of these studies the weight loss intervention consisted of bariatric surgery with associated lifestyle intervention guidelines performed (De Luis *et al* 2008b). In the other three studies obese subjects were enrolled in weight loss interventions consisting of dietary, physical activity and behavioural components that lasted either two months (De Luis *et al*. 2008a), three months (De Luis *et al*. 2006), or six months (Takakura *et al*. 2005).

The results indicated that the BMI of both the Thr54-allele carriers and the Ala54Ala homozygotes decreased from baseline to follow-up in three of the four intervention studies (De Luis *et al*. 2006, De Luis *et al*. 2008a, De Luis *et al*. 2008b). As there was no difference between the Thr54-allele carriers and the Ala54Ala homozygotes for weight or BMI at baseline or follow-up, it was concluded that the *FABP2* Ala54Thr polymorphism did not affect weight loss (Takakura *et al*. 2005, De Luis *et al*. 2006, De Luis *et al*. 2008a, De Luis *et al*. 2008b). However, the fact that actual change in BMI from baseline to follow-up within each genotype group was not compared between the genotype groups needs to be borne in mind when considering this conclusion.

When considering change in fat distribution and health variables the *FABP2* Ala54Thr polymorphism does seem to influence the outcome of weight loss interventions. For instance, only the Ala54Ala homozygotes achieved a lower fat mass after a three-month intervention (De Luis *et al*. 2006), while Takakura *et al*. (2005) reported a higher WC in Thr54-allele carriers at six-month follow-up. However, the other interventions led to a decrease in WC (De Luis *et al*. 2006, De Luis *et al*. 2008a) and fat mass (De Luis *et al*. 2008a, De Luis *et al*. 2008b) in both Thr54-allele carriers and Ala54Ala homozygotes. In Ala54Ala homozygotes the three-month intervention also effectively decreased LDL and leptin levels (De Luis *et al*. 2006), while the two-month intervention decreased blood pressure, TC, TG, insulin, leptin levels and glucose levels (De Luis *et al*. 2008a). The only positive effects found in Thr54-allele carriers were decreases in systolic blood pressure and glucose levels (De Luis *et al*. 2006), while a negative effect, namely a lower metabolic rate compared to the Ala54Ala homozygotes was reported at six-month follow-up (Takakura *et al*. 2005). In contrast to these results, the *FABP2* Ala54Thr polymorphism did not influence the significant improvements in weight, fat mass, blood pressure, glucose, total cholesterol, LDL, HDL, and TG levels at three-, nine- and 12-months following bariatric surgery (De Luis *et al*. 2008b).

In summary, it seems that the *FABP2* Ala54Thr polymorphism does not influence weight loss outcome in terms of BMI. However, the Ala54Ala homozygotes benefit more from weight loss interventions to decrease risk factors for obesity related comorbidities.

Table 2.10: The association between the *FABP2* Thr54Ala polymorphism and change in weight and other variables following a weight loss intervention.

Population	Sample size	Genotype frequencies	Inclusion criteria	Weight loss intervention	Variables measured	Results	Reference
Caucasian: Spain	n=69 ♀= 55 ♂= 14	A54A=54% A54T=30% T54T=16%	BMI>30	***3 month intervention:*** Diet = 6384 kJ/ 1520 kCal, 52% carbohydrates, 25% fat, 23% proteins. Exercise: 60 min aerobic 3×/week	***Measured at 0, 3 months:*** BMI, WC, HC, WHR, BW, FM, FFM, BP, TC, HDL, LDL, TG, Energy, carbohydrate, protein, fat intake, RMR, VO_2 max, BG, insulin, HOMA-IR, lipoprotein (a), CRP, Adipocytokines: leptin, adiponectin, resistin, TNF-α, IL-6.	No difference in weight loss or changes in any of the other variables between the T54 allele carriers and the A54A homozygotes. ***Change over 3 months within genotype groups:*** T54: ↓ BMI, WC, SBP, glucose, ↑ VO_2. A54A: ↓ BMI, fat mass, WC, LDL, leptin, ↑ VO_2.	De Luis *et al*. (2006)
Caucasian: Spain	n = 204 ♀= 154 ♂= 50	A54A=54% A54T=40% T54T=6%	BMI>30	***2 months intervention*** randomly assigned to groups: <u>Diet 1:</u> low-fat diet 1500 kcal/day, 27% fat, 52% carbohydrate, 20% protein. <u>Diet 2:</u> low carbohydrate diet 1507 kcal/day, 36% fats, 38% carbohydrates, 26% proteins. Exercise: 60 min aerobic 3×/week	***Measured at 0, 2 months:*** All as described above (De Luis *et al*. 2006)	***Change over 2 months within genotype groups:*** <u>Diet 1 & 2 (same effects):</u> A54A: ↓ BMI, weight, fat mass, WC, WHR, BP, TC, TG, insulin, leptin T54: ↓ BMI, weight, WC, fat mass ***Differences between genotype*** Diet 1: T54 = ↑ SBP, glucose, leptin (at baseline) Diet 1: T54 = ↑ SBP, leptin (at follow-up) Diet 2: T54 = ↑ WC, insulin, leptin (at baseline) Diet 2: T54 = ↑ WC, leptin (at follow-up)	De Luis *et al*. (2008a)
Caucasian: Spain	n = 41 ♀= 32 ♂= 9	A54A=56% A54T=36% T54T=7%	BMI>40	***Intervention*** Bariatric surgery: biliopancreatic diversion Follow-up = 3, 9, 12 months after surgery	***Measured at 0, 3, 9, 12 months:*** BMI, fat mass (measured with body electrical bioimpedance), BP, TC, HDL, LDL, TG, Glucose Frequency of patients with DM, hypertension, hyperlipidemia.	T54 = ↑ TG at baseline ***Within genotype group changes:*** In A54A and T54 groups = ↓ BMI, fat mass, SBP, glucose, TC, LDL, TG, ↑ HDL (at 1 yr) No association between genotype and weight loss at 3, 9 and 12 months after bariatric surgery	De Luis *et al*. (2008b)
Japanese	n = 80 ♀ only	A54A=56% A54T=37% T54T=7%	BMI>25	***6 months intervention*** Limit energy intake by 1200 kcal/day Protein intake = 70 g/day	***Measured at 0 and 6 months:*** BMI, weight & BMI at 20 years of age, body fat %, glucose, HbA1c, insulin, HOMA-IR, TC, TG, LDL, free T_3, free T_4, TSH, VWAT, SWAT, RMR	***Change over 6 months within genotype groups:*** Thr54 = ↓ adjusted RMR, ↑ WC at 6 months	Takakura *et al*. 2005

A54A = Ala54 homozygotes, A54T = heterozygotes, T54T = Thr54 homozygotes, T54 = Thr54 allele carriers, BMI = body mass index, WC = waist circumference, HC = hip circumference, WHR = waist-hip ratio, BW = body water, FM = fat mass, FFM = fat free mass, BP = blood pressure, SBP = systolic blood pressure, TC = total cholesterol, HDL = high density lipoprotein, LDL = low density lipoprotein, TG = triglycerides, RMR = resting metabolic rate, BG = blood glucose, VWAT = visceral white adipose tissue, SWAT = subcutaneous white adipose tissue.

2.4 The insulin induced gene 2 (*INSIG2*)

2.4.1 The *INSIG2* gene

The insulin induced gene 2 (*INSIG2*) is located on chromosome 2 at position 2q14.1 (Herbert *et al*. 2006), has a total length of 21548 bp and contains six exons (NCBI 2008).

2.4.2 The INSIG2 protein

The *INSIG2* gene encodes a 225 amino acid protein that was identified in a search of an Expressed Sequence Tags (EST) database for proteins homologous to the INSIG1 protein (Yabe *et al*. 2002, NCBI 2008). The amino acid sequences of the INSIG1 and INSIG2 proteins are 59% identical and differ mainly due to a much shorter NH_2-terminal sequence found in INSIG2. Both proteins contain six membrane spanning helices (Figure 2.3) and are expressed in the endoplasmic reticulums (ER) of cells (Yabe *et al*. 2002).

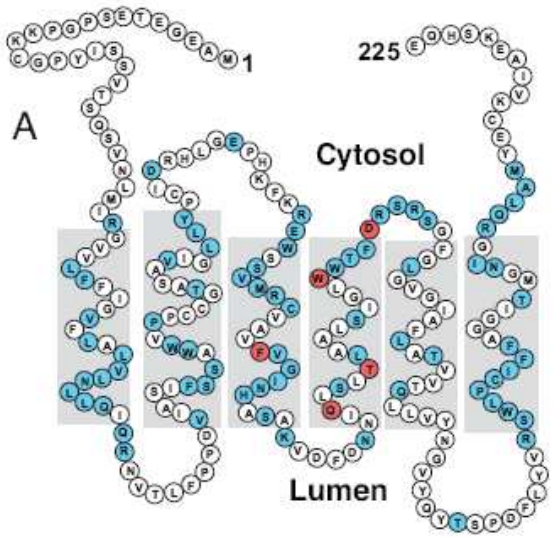

Figure 2.2: The human INSIG2 protein (from Radhakrishnan *et al*. 2007).

The INSIG1 protein is predominantly expressed in the liver of humans. Relatively low expression levels are also found in extrahepatic tissues such as the testes, kidney, brain, lung, intestine, trachea, thymus, muscle, ovaries, heart, cervix, oesophagus, placenta, prostate, adipose tissue and colon (Krapivner *et al*. 2008). Although INSIG2 is also highly expressed in the liver, it shows a more ubiquitous expression pattern in humans and is predominantly expressed in extrahepatic tissues including the adipose tissue. In comparison to INSIG1, the INSIG2 expression levels are higher in all the mentioned extrahepatic tissues (Krapivner *et al*. 2008).

2.4.3 Physiological function of INSIG2

As the functions of the INSIG1 and INSIG2 proteins overlap and the specific physiological contribution of each has not yet been fully elucidated, both INSIG proteins are discussed in this section. It should be noted that initial *in vitro* and *in vivo* research investigating the physiological role of these proteins was conducted on INSIG1 proteins only, as they were discovered in 1997 (Peng *et al*. 1997), five years before the INSIG2 proteins (Yabe *et al*. 2002).

INSIG1 and *INSIG2* are both attractive candidate genes for investigation of potential association with obesity development due to the role of their protein products in adipocyte metabolism as well as the regulation of the synthesis of cholesterol, triglycerides and other lipids through the sterol regulatory element binding protein (SREBP) pathway. Both INSIG proteins block the proteolytic activation of the SREBPs. Activated SREBPs are necessary to stimulate the transcription of more than 30 genes required for the synthesis and uptake of fatty acids, triglycerides, phospholipids, cholesterol, the NADPH cofactor and the low density lipoprotein (LDL)-receptor (Horton *et al*. 2002, DeBose-Boyd 2008).

The three SREBP isoforms, namely SREBP1a, SREBP1c and SREBP2 are encoded by two genes as inactive precursor proteins attached to the ER of cells (reviewed by Horton *et al*. 2002, Goldstein *et al*. 2006). Immediately following their synthesis, the ER attached SREBPs bind to the SREBP cleavage-activation protein (SCAP). In sterol depleted cells, SCAP transports SREBPs from the ER to the Golgi apparatus where SREBPs are processed by two membrane bound proteases (Figure 2.3). Consequently, the active SREBP portions are released into the cytosol to enter the nucleus and activate transcription by binding to the sterol response element (SRE) in the promoter/ enhancer regions of multiple target genes (Brown *et al*. 2002, Horton *et al*. 2002, Goldstein *et al*. 2006, DeBose-Boyd 2008).

For example, SREBPs increase cholesterol synthesis through direct activation of the expression of 3-Hydroxy-3-methylglutaryl (HMG)-CoA reductase, a rate-limiting enzyme required to convert HMG-CoA to mevalonate, an important step in the cholesterol biosynthesis pathway (Sul & Storch 2006, DeBose-Boyd 2008). Reversal of this increase in reductase is mediated through feedback control by the end-products of the mevalonate pathway, namely cholesterol and cholesterol derivates oxysterols (Radhakrishnan *et al*. 2007). Both cholesterol and oxysterols induce the binding of SCAP to either the INSIG1 or INSIG2 anchor proteins, but utilize different mechanisms to achieve this (Yang *et al*. 2002, Adams *et al*. 2004). Cholesterol binds directly to SCAP and consequently initiates the binding of SCAP to an INSIG protein (Adams *et al*. 2004, Radhakrishnan *et al*. 2004). However, SCAP does not bind directly to the oxysterols because they contain polar groups such as extra hydroxyl or keto groups. INSIG2 protein has high specificity for these hydroxyl groups of oxysterols and facilitates the binding of oxysterols to SCAP (Radhakrishnan *et al*. 2007). Therefore in sterol abundant cells, the binding of sterols and INSIG proteins to SCAP prevents SCAP from escorting SREBPs to the Golgi apparatus and consequently block the proteolytic activation of SREBPs (DeBose-Boyd 2008). The SREBP-SCAP complex now

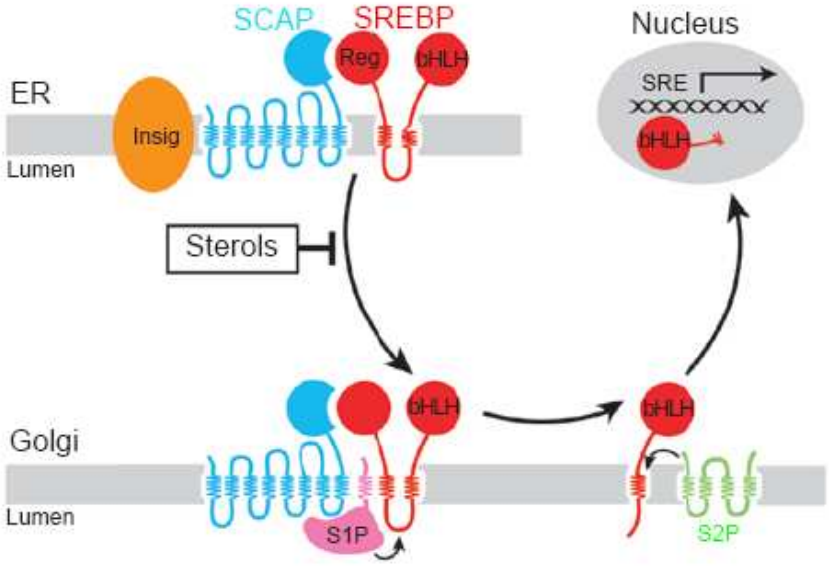

Figure 2.3: The regulation of the synthesis of cholesterol and other products by the INSIG proteins in the SREBP-SCAP pathway. Scap transports the SREBP from the ER to the Golgi apparatus, where SREBP are processed by two proteases, Site-1 protease (S1P) and Site-2 protease (S2P), to release the active bHLH-Zip domain of SREBP. The released domain migrates to the nucleus and binds to a sterol response element (SRE) in the promoter region of target genes (such as the gene encoding HMG-CoA reductase), activating their transcription. Accumulation of sterols in ER membranes stimulates the binding of Insig proteins to Scap. This prevents the translocation of SREBP to the Golgi-apparatus and the transcription of reductases is inhibited. Consequently, the production of cholesterol and other sterols are increased (DeBose-Boyd 2008).

remains in the ER and cholesterol and fatty acid syntheses are consequently inhibited, which may result in lower plasma cholesterol and triglyceride levels (Yabe *et al*. 2002, Yang *et al*. 2002, Sun *et al*. 2005, Goldstein *et al*. 2006).

The regulation of HMG-CoA reductase by the INSIG proteins has been illustrated by *in vitro* research. For example, in Chinese hamster ovary cells the overexpression of Insig-1 and Insig-2 proteins activates the degradation of HMG-CoA reductase (Sever *et al*. 2003a). It was further indicated that this degradation of HMG-CoA reductase is suppressed in human SV-589 fibroblasts when the amounts of either Insig-1 or Insig-2 are decreased through RNA interference (Sever *et al*. 2003b). However, when both Insig-1 and Insig-2 were simultaneously removed with RNA interference, no degradation of HMG-CoA reductase occurs (Sever *et al*. 2003b). These studies and others (Lee *et al*. 2005a) illustrate that the degradation of HMG-CoA reductase is dependent on the presence of the Insig-1 and Insig-2 proteins and is only stopped when both Insig proteins are removed. This might indicate that the two proteins show complementary roles in the regulation of the SREBP pathway. *In vivo* research by Engelking *et al*. (2005) have shown that Insig-1 or Insig-2 knockout mice appear normal, however only the combined knockout of both *Insig* genes was associated with a phenotype related to disturbances of the SREBP pathway. It also appears that a decrease in concentration of one Insig protein is compensated for by an increase in the other Insig protein (Engelking *et al*. 2005). However, further research by

Krapivner *et al*. (2008) showed increased mature SREBP1 protein levels and increased expression of all eight SREBP target genes under investigation following siRNA gene silencing of the *Insig1* but not the *Insig2* gene. These authors concluded that *Insig-1* may play a more profound role than *Insig-2* in the expression of SREBP protein and its target genes. More research is necessary to confirm these findings.

A four-fold increase in *Insig-1* mRNA that was observed in the fat tissue of normal rats at the onset of diet induced obesity pointed to the possibility that Insig proteins might play a role in adipocyte metabolism (Li *et al*. 2002). This observation was confirmed when mice fed a high fat diet for five weeks showed progressively increased epididymal fat pad weight, leptin and *Insig-1* mRNA levels (Li *et al*. 2003). Furthermore, restriction of food intake by 50% led to decreased expression of *Insig-1* mRNA in mice (Li *et al*. 2003). It is known that when the diet is high in total energy and fat to induce weight gain and obesity development, adipose tissue expands as a result of triglyceride accumulation in adipocytes, with preadipocytes differentiating into mature adipocytes (Peters 2006). In support of a possible role for INSIG proteins in adipocyte metabolism, *in vitro* experiments have shown a rise in *Insig-1* and *Insig-2* mRNA during maturation of 3T3-L1 preadipocytes (Li *et al*. 2003, Krapivner *et al*. 2008). Both studies indicated that the rise in Insig-2 expression was more pronounced than Insig-1 expression (Krapivner *et al*. 2008). This together with the fact that the INSIG2 protein is the predominant isoform expressed in adipose tissue may indicate that the *INSIG2* gene may play a more profound role in controlling adipocyte metabolism and contribute to BMI changes. It was further illustrated that the transfection of the 3T3-L1 preadipocytes with mouse or human *Insig-1* cDNA prevents the accumulation of lipids in differentiating preadipocytes and inhibits lipogenesis completely (Li *et al*. 2003). Further *in vivo* work confirmed that the overexpression of *Insig-1* or *Insig-2* in the livers of Zucker diabetic fatty rats (ZDF (fa/fa) rat model) reduced SREBP activation of target lipogenic enzymes. This consequently resulted in a reduction in lipogenesis, hypertriglyceridemia and hepatic steatosis independent of food intake, body weight and fat weight (Takaishi *et al*. 2004). It is thus proposed that SREBPs play a role in the promotion of adipogenesis and triglyceride storage while the INSIG proteins potentially block these actions of the SREBPs. Based on these premises it was postulated that in individuals with altered INSIG activity the subsequent elevation in plasma triglyceride levels and storage in adipose tissue could be a plausible explanation for a possible association with obesity development (Yabe *et al*. 2002, Herbert *et al*. 2006).

In summary, INSIG2 blocks cholesterol and fatty acid synthesis by binding to the SCAP and preventing translocation and activation of SREBP and consequently decreases the transcription of necessary genes for these biosynthesis pathways. In adipocyte metabolism, INSIG2 expression seems to increase following a high fat diet and consequently limits adipocyte differentiation and lipogenesis.

2.4.4 The rs7566605 polymorphism near *INSIG2*

The rs7566605 polymorphism near *INSIG2* was the first common obesity associated gene variant to be identified through a GWA approach using participants from the Framingham Heart Study (Herbert *et al*. 2006). The

mutation produces a Guanine (G) to Cytosine (C) transition which is located 10 kb upstream of the transcription start site of the *INSIG2* gene (Herbert *et al*. 2006).

Whether this variant itself, or maybe another variant in strong linkage disequilibrium (LD) alters *INSIG2* expression, is still unknown. It was pointed out that it is unlikely that the *INSIG2* rs7566605 polymorphism is functional due to its location and that this mutation may rather be a marker for the yet unidentified functional variation elsewhere in the *INSIG2* gene or another gene at the same locus (Smith *et al*. 2007). However, Hotta *et al*. (2008a) argued that the *INSIG2* rs7566605 polymorphism may affect the transcriptional activity of *INSIG2* and may therefore have an impact on its expression levels (Hotta *et al*. 2008a). More research is necessary to elucidate these findings.

It has been shown that a variant INSIG2 protein lost the ability to bind with SCAP and consequently decreasing SREBP activitation and the transcription of biosynthetic enzymes (Gong *et al*. 2006). In contrast, others have hypothesized that a polymorphism may result in *INSIG2* overexpression in presence of excess sterols and consequently block cholesterol synthesis through the SREBP pathway to a greater degree (Oki *et al*. 2009). However, these findings still need to be confirmed by functional analyses of the polymorphism and further *in vivo* and *in vitro* investigations.

2.4.5 Genotype and allele frequencies

The frequency of the wild-type GG genotype of the *INSIG2* rs7566605 polymorphism reported for Caucasian populations with European ancestry ranges between 43 to 50%, but is much higher in African Americans at 59 to 62% and American Indians at 62% (Table 2.11). Consequently, the frequency of the mutant CC genotype ranges between 10 and 13% in Caucasians but only between 5.3 to 5.9% in African Americans.

In Caucasian populations the minor allele frequency of the *INSIG2* rs7566605 polymorphism ranges from 0.31 to 0.37. This range is similar in Japanese and other populations of Eastern origin but lower in African-American and Native-American populations.

Table 2.11: Genotype frequencies of the rs7566605 polymorphism near *INSIG2* and frequency of the mutant C-allele.

Population	Disease state	n	GG %	GC %	CC %	C-allele %	References
Caucasian							
Denmark	Controls	5106	44	45	11	33	Andreasen *et al*. 2008
	Overweight	6973	45	44	11	33	
	Obese	4702	45	44	11	33	
UK		1372	50	42	8	31	Hall *et al*. 2006
UK: Epic cohort		4916	46	44	10	32	Loos *et al*. 2007
MRC Ely study		1683	47	42	11	32	
FHS offspring		694	-	-	-	37	Herbert *et al*. 2006
UK		2721	48	42	10		Smith *et al*. 2007
UK		566			10	33	Smith et al. 2007
UK: 77% Caucasian	DM2	747	45	44	11		Smith et al. 2007

Table 2.11: *continued*

Population	Disease state	n	GG %	GC %	CC %	C-allele	References
Caucasian							
USA: 56% Caucasian	Overweight	1994	45	44	11		Franks *et al*. 2008
USA: >97% Caucasian	Morbidly obese	707	43	44	13	35	Chu *et al*. 2008
Canada	MetS	66	47	44	9	31	Pollex *et al*. 2007
	Without MetS	165	45	48	6	30	
USA & Ireland		752	47	44	9	31	Orkunoglu-Suer *et al*. 2008
USA	BMI>33	1026	45	45	10		Boes *et al*. 2008
	Controls	818	44	44	12		
Austria		1696	43	45	12		Boes *et al*. 2008
Germany		4089	46	44	10		Rosskopf *et al*. 2007
Germany	Obese children	293	50	43	7		Reinehr *et al*. 2008
Germany	Obese children	280	52	41	7		Reinehr *et al*. 2009
Germany (KORA)		4084	43	46	11		Lyon *et al*. 2007
Scandinavia		913	44	45	11		Lyon *et al*. 2007
Iceland		5187	43	45	12		Lyon *et al*. 2007
Sweden		634				36	Krapivner *et al*. 2008
FHS		8359	46	42	12		Lyon *et al*. 2007
Italy		925				24	Ciullo *et al*. 2008
Hispanic American							
Hispanic American	Overweight	591	50	41	9.0		Franks *et al*. 2008
African							
African American		893	62	32	6		Lyon *et al*. 2007
African American	Overweight	713	59	34	5		Franks *et al*. 2008
Afro-Caribbean:		69			12	29	Smith *et al*. 2007
Native American							
American Indian	Overweight	85	62	33	5		Franks *et al*. 2008
Canada: Kivalliq Inuit	MetS	13	77	15	8	15	Pollex *et al*. 2007
	Without MetS	115	55	37	8	26	
Canada: Greenland Inuit	MetS	163	62	36	2	20	Pollex *et al*. 2007
	Without MetS	930	65	30	5	20	
Canada: Oji-Cree	MetS	156	63	33	4	20	Pollex *et al*. 2007
	Without MetS	270	58	37	5	23	
Asian							
Japanese		2233	47	43	10	32	Kuzuya *et al*. 2007
Japanese	Obese	908	46	40	14	34	Hotta *et al*. 2008a
	Controls	1495	46	44	9	31	
Japanese		1976	43	44	13	35	Tabara *et al*. 2008
American Japanese		885	46	43	11	32	Oki *et al*. 2009
Japanese		378	39	50	11	36	
Chinese		3125	40	47	12	36	Yang *et al*. 2008
Chinese	Obese	258	39	52	9	35	Zhang *et al*. 2008
	Controls	982	48	43	9	31	
American from Asia/ Pacific	Overweight	150	40	45	15		Franks *et al*. 2008
Chinese Canadian	MetS	57	46	37	18	36	Pollex *et al*. 2007
	Without MetS	236	40	48	12	36	
Chinese: school children & adolescents	Normal weight	607	42	44	14		Wang *et al*. 2008
	Overweight	725	42	44	14		
	Obese	708	42	44	14		
India: AIIMS cohort	CAD (n=255)	610	51	42	7	28	Kumar *et al*. 2007
IGV cohort		1577	49	42	9	30	
UK Indians		103			17	36	Smith *et al*. 2007
South Asian Canadian	MetS	100	58	32	10	26	Pollex *et al*. 2007
	Without MetS	214	56	38	7	25	

UK: United Kingdom; MRC: Medical Research Council; UDACS: University College London Diabetes and Cardiovascular Disease Study; USA: United States of America; FHS: Framingham Heart Study.

2.4.6 Associations with obesity related phenotypes

Association with weight, BMI and obesity

Following the first identification of an association between the *INSIG2* rs7566605 polymorphism and BMI using a genome wide association (GWA) approach on 694 individuals from the Framingham Heart Study population (Herbert *et al*. 2006), two further independent GWA scans in Caucasian Americans (Liu *et al*. 2008) and South Italians (Ciullo *et al*. 2008) confirmed these results (Table 2.12).

Herbert *et al*. (2006) further investigated their GWA results using samples from cohort studies and family based studies. In four of the five studies obesity was associated with a higher frequency of CC homozygotes in Caucasian subjects from Germany, Poland and the United States of America as well as with African-Americans. The results of these analyses indicate that the recessive model (CC homozygous genotype compared to the GC and GG genotypes) is associated with a higher BMI, with individuals who are homozygous for the C-allele having a 1 kg/m^2 higher BMI compared to G-allele carriers (Herbert *et al*. 2006). In the meta-analysis of 9881 individuals identified from all the case-control samples in the cohort and family based studies, Herbert *et al*. (2006) indicated that individuals homozygous for the mutant C-allele incurred a 1.22-fold increased risk of being obese compared with individuals with the G-allele. The association with BMI was further confirmed by Lyon *et al*. (2007) in an analysis comprising nine independent large cohorts studies with nearly 17000 individuals across multiple ethnicities, showing positive associations in five of the nine cohorts including Caucasians from the United States of America, Iceland, Germany, the Framingham Heart Study and Costa Ricans with an American-Indian and Spanish ancestry. Rosskopf *et al*. (2007) also reported a higher risk for obesity amoung CC homozygotes from Germany. In populations with Eastern ancestry the CC genotype or C-allele carriers was also associated with severe obesity in Japanese (Hotta *et al*. 2008a) and with an increased risk for obesity and a higher BMI in Chinese (Yang *et al*. 2008, Zhang *et al*. 2008).

To illustrate heritability of these mutations and associations with weight status Herbert *et al*. (2006) tracked the presence of the *INSIG2* rs7566605 polymorphism in a Caucasian Western European parent-child trios sample. The trios comprised both parents and one obese child or adolescent offspring and assessments involved the detection of an allele transmitted from the obese parent to the obese offspring. The results show that the *INSIG2* rs7566605 polymorphism was associated with obesity from an early age, which was explained by the observed overtransmission of the C-allele to the obese offspring. However, in a further study by Dina *et al*. (2007a) in French Caucasian trios this association could not be confirmed.

Despite these positive associations, several other independent studies have failed to observe an association between the *INSIG2* rs7566605 polymorphism and obesity or BMI in Caucasians from the United Kingdom (Hall *et al*. 2006, Loos *et al*. 2007, Smith *et al*. 2007), Austria (Boes *et al*. 2008), France (Dina *et al*. 2007a), Denmark (Andreassen *et al*. 2008, Vimaleswaran *et al*. 2009), Italy (Ciullo *et al*. 2008), Sweden (Krapivner *et al*. 2008), Belgium (Peeters *et al*. 2009), Scandinavia (Lyon *et al*. 2007) and the United States of America (Boes *et al*. 2008,

Orkunoglu-Suer *et al*. 2008). The lack of association was also evident in other populations such as Afro-Caribbeans (Smith *et al*. 2007), Indians (Smith *et al*. 2007, Kumar *et al*. 2007), Asians (Feng *et al*. 2007) the Japanese (Tabara *et al*. 2008, Oki *et al*. 2009, Kuzuya *et al*. 2007), Japanese-Americans (Oki *et al*. 2009), Filipino women (Marvelle *et al*. 2008) and Canadian populations, including the Kivalliq Inuit, Greenland Inuit, Oji-Cree and those of South Asian, Chinese and European descent (Pollex *et al*. 2007). More specifically, the work by Loos *et al*. (2007) in actual fact points to an association in the opposite direction, namely CC homozygotes appearing to be leaner than individuals carrying the G-allele, versus the observation by Herbert *et al*. (2006) that the leaner individuals carried the G-allele. Kumar *et al*. (2008) also reported that the CC genotype was more frequent in the non-obese when compared to the obese Indian population.

Several authors suggested that the different BMI distributions in the populations under investigation may explain the inconsistency in the association of BMI with the *INSIG2* rs7566605 polymorphism (Herbert *et al*. 2006, Hall *et al*. 2006, Lyon *et al*. 2007, Smith *et al*. 2007). These authors have particularly found a lack of association when a population had lower overweight and obesity levels and the BMI was distributed more in the normal weight range. Therefore, they suggested that a population with low obesity prevalence may lack sufficient power to investigate the association between the *INSIG2* rs7566605 polymorphism and obesity. According to Kuzuya *et al*. (2007) this may be the case in the Japanese where only 0.97% of male and 3.37% women in the sample were obese and no association with BMI was reported. Herbert *et al*. (2006) have also shown that if the upper quartile BMI values are excluded from their analysis in the German population, the evidence for an association between the CC genotype of the *INSIG2* rs7566605 polymorphism and BMI was eliminated. Rosskopf *et al*. (2007) reported no association between the *INSIG2* rs7566605 polymorphism and BMI in a population-based sample, however after manipulating their data by excluding all the normal weight BMIs from the population-based sample and only including overweight BMIs in the analysis, the CC homozygotes had a higher BMI than the G-allele carriers. These authors therefore conclude that the association between the *INSIG2* rs7566605 polymorphism and obesity is strongest in those who are more obese (Herbert *et al*. 2006, Rosskopf *et al*. 2007). Whether this hypothesis is true and the possible physiological explanation thereof still needs to be elucidated.

In summary, the majority of studies that did find an association between the *INSIG2* rs7566605 polymorphism and obesity or BMI support the possibility that being a CC homozygote or having the C-allele predispose an individual to a higher BMI and obesity risk.

Table 2.12: The association between the rs7566605 polymorphism near *INSIG2* and weight, BMI and obesity.

Population		n	Design	Association with obesity		Association with BMI		Reference
				Yes	No	Yes	No	
Caucasian								
FHS		694	Family based offspring cohort	obese = ↑ CC		GWA: CC = ↑ BMI	-	Herbert *et al*. 2006
Germany		3996	Cohort with case-control analysis	obese = ↑ CC		CC = ↑ BMI	-	Herbert *et al*. 2006
Germany		4089	Cross-sectional	-	✓	-	✓	Rosskopf *et al.* 2007
Germany	Overweight	2701	Overweight sub-sample of cross-sectional sample	CC = ↑ obesity risk		CC = ↑ BMI	-	Rosskopf *et al.* 2007
Poland and USA		2761	Case-control	obese = ↑ CC		-	-	Herbert *et al*. 2006
USA: >95% Caucasian		2726	Nurses health study (lower mean BMI compared to other samples)	-	-		✓	Herbert *et al*. 2006
Western-Europe		1104	Family: Parents + obese child or adolescent	C-allele = transmitted to obese child		C-allele = ↑ BMI from early age		Herbert *et al*. 2006
Caucasian + African American		9881	Meta-analysis of case-control samples	obese = ↑ CC	-	-	-	Herbert *et al*. 2006
Denmark		16781	Population based samples with case-control analysis	-	✓	-	✓	Andreasen *et al*. 2008
Denmark		3878	Sub-sample of population based sample	-	-	-	✓	Andreasen *et al*. 2008
Belgium	obese controls	1078 323	Case-control		✓		✓	Peeters *et al.* 2009
Austria		1696	Population sample with case-control analysis		✓		✓	Boes *et al*. 2008
Canada		231	Population sample	-	-		✓	Pollex *et al*. 2007
USA	BMI>33 Controls	1026 818	Case-control		✓		✓	Boes *et al*. 2008
USA and Ireland		752	Cohort from populations at 8 centres	-	-		✓	Orkunoglu-Suer *et al*. 2008
USA		1000	Population	GWA: CC = ↑ obesity	-	-	-	Liu *et al*. 2008
UK		1372	Family based study with 4-6 members of a family with a hypertensive proband	-	✓	-	✓	Hall *et al*. 2006
UK		4916 1683 6599	Cohort: EPIC 5000 Cohort: MRC Ely Combined cohorts	-	-	C-allele =↓ BMI	 ✓ ✓	Loos *et al*. 2007
UK		3012 572	Cohort: ♂ Cohort: Cross-sectional of DM2 pt.		✓ ✓		✓ ✓	Smith *et al*. 2007
Iceland		5187	Cohort	obese = ↑ CC		CC = ↑ BMI		Lyon *et al.* 2007
Germany		4084	Cohort: KORA S3		✓		✓	Lyon *et al.* 2007

Table 2.12: *continued*

Population	n	Design	Association with obesity		Association with BMI		Reference
			Yes	No	Yes	No	
Caucasian							
Essen	1381	Cohort	obese = ↑ CC	-	ni	ni	Lyon *et al.* 2007
France	1531	Case-control: Obese vs. control children	-	✓	ni	ni	Dina *et al.* 2007a
France	1138	Children of family study with at least 1 obese sibling	-	C-allele not transmitted	-	✓	Dina *et al.* 2007a
France	7593	Case-control: meta-analysis from: cohort, family (parents of obese child) unrelated adult case-control sample	-	✓	-	-	Dina *et al.* 2007a
	4998		-	✓	-	✓	
	1572		-	✓	-	-	
	1023		-	✓	-	-	
USA: >97% Caucasian	707	Intervention: Morbidly obese	ni	ni	-	✓	Chu *et al.* 2008
USA: 56% Caucasian Overweight	3533	RCT	ni	ni	-	✓	Franks *et al.* 2008
European American	-	DB-RCT: schizophrenia pt	ni	ni	-	✓	Skelly *et al.* 2007
Danish & Estonian children	2003	School based cross-sectional study	-	✓		✓	Vimaleswaran *et al.* 2009
Scandinavia	512	Family: siblings without DM	ni	ni	-	✓	Lyon *et al.* 2007
Scandinavia	913	Cohort	-	✓	-	✓	Lyon *et al.* 2007
Sweden	634	Cohort of healthy men	ni	ni	-	✓	Krapivner *et al.* 2008
USA	1224	Family: (CAMP) children with asthma history and their parents	ni	ni	CC = ↑ BMI		Lyon *et al.* 2007
Italy	925	Populations in Campora & Gioi, remote South Italy	-	✓	-	✓	Ciullo *et al.* 2008
FHS exam 1	1491	Cohort	-	✓	-	✓	Lyon *et al.* 2007
FHS exam 2	1267		-	✓	-	✓	
FHS exam 3	1310		obese = ↑ CC		-	✓	
FHS exam 4	1431		-	✓	-	✓	
FHS exam 5	1431		-	✓	-	✓	
FHS exam 6	1429		-	✓	-	✓	
African							
African American	866	Family based sample	obese = ↑ CC	-	ni	ni	Herbert *et al.* 2006
African American	398	Case-control	obese = ↑ CC	-	ni	ni	Herbert *et al.* 2006
African American	893	Cohort	-	✓	-	✓	Lyon *et al.* 2007
African American	-	DB-RCT: schizophrenia pt (n=756 with European American)	ni	ni	-	✓	Skelly *et al.* 2007
UK: Afro-Caribbean	70	Cohort: Cross-sectional of DM2 pt	-	✓	-	✓	Smith *et al.* 2007

Table 2.12: *continued*

Native American								
Canadian Greenland Inuit		1093	Population sample	ni	ni	-	✓	Pollex *et al*. 2007
Canadian Kivalliq Inuit		128	Population sample	ni	ni	-	✓	Pollex *et al*. 2007
Canadian Oji-Cree		426	Population sample	ni	ni	-	✓	Pollex *et al*. 2007
American-Indian + Spanish		1284	Family: Costa-Rica cohort of children with asthma + families	ni	ni	CC = ↑ BMI	-	Lyon *et al.* 2007
Asian								
Filippines		1886	Population sample of women	ni	ni	-	✓	Marvelle *et al*. 2008
China		2040	School-based study with selected obese, overweight, normal weight children and adolescents	-	✓	-	✓	Wang *et al*. 2008
China		3125	Combined cross-section sample of:	-	✓	-	✓	Yang *et al*. 2008
		1574	Cross-sectional Beijing sample	-	✓	-	✓	
		1551	Cross-sectional Shanghai sample	CC = ↑ risk for obese or overweight	-	CC = ↑ BMI	-	
China: Uyghurs		1240	Population sample: Uyghur farmers	Obese = ↑ C-allele	-	C-allele=↑ BMI	-	Zhang *et al*. 2008
India		1577	Population-based cohort with case-control analysis.	-	✓	ni	ni	Kumar *et al*. 2007
India		610	Case-control: all	Obese = ↓ CC	-	-	✓	Kumar *et al*. 2007
		255	CAD cases	-	✓	-	✓	
		355	controls	Obese = ↓ CC	-	-	✓	
Indian in UK		105	Cohort: Cross-sectional of DM2 pt	-	✓	-	✓	Smith *et al*. 2007
South Asians in Canada		314	Population sample	ni	ni	-	✓	Pollex *et al*. 2007
Japan		1976	Population sample	-	✓	-	✓	Tabara *et al*. 2008
				-	✓	-	✓	
Japan		2233	Population sample	-	✓	-	✓	Kuzuya *et al*. 2007
Japanese Americans		885	Cross-sectional of an epidemiological study	ni	ni	-	✓	Oki *et al*. 2009
Japan		378				-	✓	
Chinese Canadians		293	Population sample	ni	ni	-	✓	Pollex *et al*. 2007
Japan:	Obese	908	Case-control	obese = ↑ CC	-	-	✓	Hotta *et al*. 2008a
	Controls	1495				-	✓	

*Recessive model = compares the CC homozygotes with G-allele carriers; Dominant model = compares GG homozygotes with C-allele carriers

EPIC: European Prospective Investigation of Cancer; MRC: Medical Research Council; NILS-LSA: National Institute for Longevity Sciences – Longitudinal Study of Aging; CAMP: Childhood Asthma Management Program;

Measures of body fat distribution and content

Only three studies thus far have reported a positive association between the *INSIG2* rs7566605 polymorphism and fat distribution (Table 2.13). In Caucasian populations it was indicated that the C-allele carriers had a higher subcutaneous adipose tissue area (Franks *et al*. 2008) and subcutaneous fat volume (Orkunoglu-Suer *et al*. 2008) than GG homozygotes. However, in contrast with the general association of the C-allele with a higher BMI, Pollex *et al*. (2007) reported a lower waist circumference in C-allele carriers in non-Caucasian populations.

Table 2.13: The association between the rs7566605 polymorphism near *INSIG2* and measures of body fat distribution and content.

Population	Fat distribution	No associations	Reference
Caucasian			
Denmark	-	WC	Andreasen *et al*. 2008
UK	-	WHR, leptin	Hall *et al*. 2006
USA: 56% Caucasian	C-allele = ↑ SAT	WC, VAT	Franks *et al*. 2008
USA & Ireland	C-allele ♀ = ↑ sc fat volume	sc fat volume in ♂	Orkunoglu-Suer *et al*. 2008
Danish & Estonian children		WC, sum of 4 skinfolds	Vimaleswaran *et al*. 2009
Belgium		WC, WHR, VFA, SFA, TFA	Peeters et al. 2009
Austria	-	WC, WHR, % body fat, vc & sc abdominal fat	Boes *et al*. 2008
Canadian	-	WC	Pollex *et al*. 2007
Native American			
Canadian: Kivalliq Inuit	-	WC	Pollex *et al*. 2007
Canadian: Greenland Inuit	-	WC	Pollex *et al*. 2007
Canadian: Oji-Cree	♀ C-allele carriers = ↓ WC	-	Pollex *et al*. 2007
Asian			
Canadian: South Asian	♀ C-allele carriers = ↓ WC	-	Pollex *et al*. 2007
Filippines	-	WC, % body fat	Marvelle *et al*. 2008
Chinese-Canadian	♂ C-allele carriers = ↓ WC	-	Pollex *et al*. 2007
China	-	WC	Yang *et al*. 2008
China	-	WHR	Zhang *et al*. 2008
Chinese: children and adolescents	-	WC, WHR, % body fat	Wang *et al*. 2008
Japan	-	Adiponectin, resistin	Tabara *et al*. 2008
Japan	-	WC, HC, WHR, fat mass	Kuzuya *et al*. 2007
Japanese Americans Japan	-	% body fat, WC	Oki *et al*. 2009
Meta-analysis			
Meta-analysis of Canadian populations inkling: Oji-Cree, Greenland Inuit, Kivalliq Inuit, South Asian, Chinese and Caucasians	C-allele carriers = ↓ WC	-	Pollex *et al*. 2007

VFA = visceral abdominal fat area; HC = hip circumference; WC = waist circumference; WHR = waist-hip-ratio; SFA, subcutaneous abdominal fat area; TFA = total abdominal fat area; PCOS = polycystic ovary syndrome.

2.4.7 Associations with health indicators

Indicators of glucose and insulin homeostasis

Although nine studies have investigated thus far an association between the *INSIG2* rs7566605 polymorphism and blood glucose and/or insulin levels in 15 populations, none could find any significant results (Table 2.14). There was also no indication of an association with type-2 diabetes prevalence, duration of type-2 diabetes, insulin resistance as measured with HOMA-IR and MetS. However, a meta-analysis of six Canadian populations with different ancestries showed that C-allele carriers had a decreased MetS risk, based on the IDF criteria for the diagnosis of this syndrome (Pollex *et al*. 2007). This finding is not in line with the proposed association of the CC genotype or the C-allele with obesity and a higher BMI.

Blood lipid profile and blood pressure

Only three studies thus far have indicated an association between the *INSIG2* rs7566605 polymorphism and blood lipid profile (Table 2.14). It was shown that obese Chinese children and adolescents with the CC genotype have higher plasma triglyceride levels than the G-allele carriers (Wang *et al*. 2008). Canadian Oji-Cree female C-allele carriers had higher LDL than GG homozygotes (Pollex *et al*. 2007). However, eight of the 11 studies summarized in Table 2.15 found no association between the *INSIG2* rs7566605 polymorphism and plasma levels of total cholesterol, triglycerides, LDL, HDL, free fatty acids or blood pressure. Further conflicting results indicate that female Japanese-Americans with the CC genotype have decreased dyslipidemia and hypercholesterolemia (Oki *et al*. 2009).

Table 2.14: The association between the rs7566605 polymorphism near *INSIG2* and indicators of glucose and insulin homeostasis, blood lipid profile and blood pressure.

Population	Glucose and insulin homeostasis		Blood lipids and blood pressure (BP)		Reference
	Association	No association	Association	No associations	
Caucasian					
Caucasians: Denmark	-	insulin, glucose after OGTT	-	TG, HDL	Andreasen *et al*. 2008
UK (>77% Caucasian)	-	DM2, duration of DM2	-	CHD	Smith *et al*. 2007
USA (>97% Caucasian)	ni	ni	-	TG, TC, HDL, LDL	Chu *et al*. 2008
USA	ni	ni	-	TC, TG, LDL, HDL, FFA	Boes *et al*. 2008
Canada	-	Glucose, MetS	-	TC, TG, LDL, HDL, BP	Pollex *et al*. 2007
Austria	ni	ni	-	TC, TG, LDL, HDL, FFA	Boes *et al*. 2008
Danish & Estonian children	ni	ni	-	TC, TG, LDL, HDL	Vimaleswaran *et al*. 2009
Native American					
Canadian Greenland Inuit	-	Glucose, MetS	-	TC, TG, LDL, HDL, BP	Pollex *et al*. 2007
Canadian Kivalliq Inuit	-	Glucose, MetS	-	TC, TG, LDL, HDL, BP	Pollex *et al*. 2007
Canadian Oji-Cree	-	Glucose, MetS	C-allele ♀ = ↑ LDL	TC, TG, HDL, BP	Pollex *et al*. 2007
Asian					
Chinese: children & adolescents	-	glucose, insulin	obese CC = ↑ TG	TC, LDL, HDL, BP	Wang *et al*. 2008
Chinese	-	Glucose	-	TG, TC, BP	Zhang *et al*. 2008
Chinese Canadian	-	Glucose, MetS	-	TC, TG, LDL, HDL, BP	Pollex *et al*. 2007
Japanese	-	BG, HOMA-IR	-	HDL, TG	Tabara *et al*. 2008
Japanese	-	DM2, glucose, insulin, HbA_1C, HOMA-IR	ni	ni	Kuzuya *et al*. 2007
Japanese	-	Insulin, HOMA-IR	-	TG, TC, HDL	Oki *et al*. 2009
Japanese Americans	-	Insulin, HOMA-IR	♀: CC = ↓ dyslipidaemia & hypercholesterolaemia	TG, TC, HDL	Oki *et al*. 2009
Japanese	-	glucose, HbA_1C	-	TC, TG, HDL, BP	Hotta *et al*. 2008a
Canadian South Asian	-	Glucose, MetS	-	TC, TG, LDL, HDL, BP	Pollex *et al*. 2007
Meta-analysis					
Meta-analysis of Canadian populations inkling: Oji-Cree, Greenland Inuit, Kivalliq Inuit, South Asian, Chinese and Caucasians	C-allele carriers = ↓ MetS risk with IDF criteria C-allele carriers ♀ = ↓ MetS risk with IDF criteria	MetS with NCEP ATPIII criteria MetS in ♂ with IDF criteria	ni	ni	Pollex *et al*. 2007

TG = triglycerides, TC = Total Cholesterol, HDL = High density lipoprotein, MetS = Metabolic Syndrome, IDF = International Diabetes Federation, NCEP ATPIII = National Cholesterol Education Program Adult Treatment Panel III.

2.4.8 Associations with weight loss outcomes

Three studies (Table 2.15) have thus far investigated the association between the *INSIG2* rs7566605 polymorphism and weight loss after a weight loss intervention programme. Two studies were executed on obese children aged six to 16 years in Germany (Reinehr *et al*. 2008, Reinehr *et al*. 2009). In both reports the children were enrolled in the one year "Obeldicks" outpatients' intervention programme comprising of nutrition education, physical exercise, behaviour therapy (covered in group sessions) and individualized psychological care components. In both studies the CC homozygotes lost less weight than G-allele carriers after following the one year intervention programme (Reinehr *et al*. 2008, Reinehr *et al*. 2009). It was further illustrated that although the total sample lost weight after one year, when considering the *INSIG2* rs7566605 polymorphism, it was only the G-allele carriers who lost weight while the CC homozygotes actually gained weight during this period (Reinehr *et al*. 2009). However, Franks *et al*. (2008) reported opposite findings for overweight adults enrolled in an ongoing Diabetes Prevention Program in the United States of America. In this randomized controlled trial, adults were assigned to one of four treatment groups and follow-up measurements were reported after one year on the programme. In the overall study the lifestyle intervention component was the most effective treatment strategy to induce weight loss compared to the placebo, Metformin and Troglitazone groups. When the *INSIG2* rs7566605 polymorphism was considered, the CC homozygotes lost more weight than the G-allele carriers after one year lifestyle intervention.

Two further studies that were not weight loss interventions, but investigated the effect of physical activity on subcutaneous fat volume and BMI, need to be mentioned. Orkunoglu-Suer *et al*. (2008) tested the influence of a 12 weeks resistant training programme on the change in subcutaneous fat volume of a trained and untrained arm of healthy Caucasian subjects. Subjects received two 45 to 60 minute sessions per week for 12 weeks. Sessions consisted of resistant training exercises for the biceps and triceps muscles in the non-dominant arm. After the 12 week intervention, the GG homozygotes experienced a decrease in subcutaneous fat levels in their non-dominant arm, while C-allele carriers were resistant to such fat losses and actually gained subcutaneous fat (Orkunoglu-Suer *et al*. 2008). Andreasen *et al*. (2008) compared the BMI of physically active and passive subjects according to the *INSIG2* rs7566605 polymorphism. Physically passive subjects had a higher BMI than physically active, which was more pronounced in the CC physically passive homozygotes who had a 1.00 unit higher BMI than the physically active CC homozygotes. In physically passive subjects, the CC homozygotes had a 0.54 unit higher BMI than the G-allele carriers (Andreasen *et al*. 2008).

Although the results seem conflicting, two of the three weight loss studies, the one exercise intervention study and the one study comparing habitual exercise all indicate that the *INSIG2* rs7566605 polymorphism may influence weight management. These studies point to the possibility that CC homozygotes may find it more difficult to lose weight on weight loss intervention programmes or with physical activity interventions.

Table 2.15: The association between the rs7566605 polymorphism near *INSIG2* and change in weight (BMI) and other variables following a weight loss intervention.

Population	Sample size	Genotype frequencies	Inclusion criteria	Weight loss intervention	Variables measured	Results	Reference
USA: 56% white 20% African-American 17% Hispanic 4% Asian 3% American Indian	n=3533	GG=49% CG=41% CC=10%	Non-DM, overweight	DPP is ongoing RCT, here results of 1 year intervention reported, pt received 1 of the following 4 interventions: 1) Placebo, 2) Metformin, 3) Troglitazone 4) Lifestyle modification programme with aim =150 min PA/wk & 7% weight loss	Weight, WC, abdominal adipose tissue distribution, lipids, insulin, glucose, OGTT	After 1 year CC = lost more weight than G-allele carriers in lifestyle intervention group No significant differences in other groups CC = lost more subcutaneous and visceral adipose areas	Franks *et al*. 2008
German children	n=293 ♀=161 ♂=132 Follow-up: n=185	GG=50% CG=43% CC=7%	obese children 6-16 yrs	1 year "Obeldicks" program: First 3 months = eating behaviour &nutrition course in 6 group sessions of 1.5h each for children and 6 parents group sessions. Months 3-9 = individual psychological family therapy Months 9-12: individual care if necessary Throughout year: 1-2/week exercise Diet: 30% fat, 15% protein, 55% carbohydrate including 5% sugar	BMI, TG, HDL, LDL, glucose, insulin, HOMA-IR, BP.	n=77 drop-out in motivation phase, n=31 drop-out in first 3 months CC=lost less weight than G-allele carriers	Reinehr *et al*. 2008
German children	n=280 ♀=154 ♂=126 Follow-up: n=240	GG=52% CG=41% CC=7%	obese children 6-16 yrs	as described above	BMI	n=40 drop-out After 1 yr on programme: Total sample = ↓ BMI by 0.71 kg/m^2 & 68% lost weight CC = ↑ BMI & G-allele carriers = ↓ BMI CC=loss less weight than G-allele carriers *INSIG* CC & *FTO* AA = highest ↑ BMI	Reinehr *et al*. 2009

Non-DM = non-diabetics, DPP = diabetes prevention programme, RCT = randomized controlled trial, PA/wk = physical activity per week, BMI = body mass index, WC = waist circumference, BP = blood pressure, HDL = high density lipoprotein, LDL = low density lipoprotein, TG = triglycerides, HOMA-IR = Homeostasis model assessment of insulin resistance, OGTT = oral glucose tolerance test.

2.5 The Fat mass and obesity associated (*FTO*) gene

2.5.1 The *FTO* gene

Peters *et al*. (1999) originally cloned the mouse *Fto* gene from screening a cDNA library of the fused toes (ft) mutant mouse whose phenotype was created by insertional mutagenesis (Van der Hoeven *et al*. 1994). This resulted in a 1.6 Mb deletion of six genes including the *Fto* gene on the mouse chromosome 8 (Peters *et al*. 1999, Peters *et al*. 2002). Following a GWA study, the human *FTO* gene was assigned to chromosome 16 at position 16q12.2 (Frayling *et al*. 2007). *FTO* has a total length of 410509 bp and contains nine exons (NCBI 2009).

2.5.2 The FTO protein

It has been proposed that the *FTO* gene encodes a 505 amino acid protein (Peters *et al*. 1999). A recent sequence similarity search revealed that the human FTO protein shares sequences homologues with the non-heme ferrous iron (Fe(II))- and 2-oxoglutarate (OG)-dependent oxygenase (non-heme dioxygenase superfamily) proteins of several eukaryotic species (Sanchez-Pulido & Andrade-Navarro 2007). Conserved amino acids involved in the binding of iron, an essential co-factor as well as 2-OG, a co-substrate of these dioxygenases, were found on the human FTO protein (Sanchez-Pulido & Andrade-Navarro 2007). These findings are supported by bioinformatic analysis that confirmed that FTO shares sequence motifs with the Fe(II)- and 2-OG dependent oxygenases (Gerken *et al*. 2007). Although the FTO protein structure is still unknown, these studies have now provided evidence that FTO might be a 2-OG dependent nucleic acid demethylase.

The *FTO* gene is expressed mostly inside the cell nucleus of almost all human tissues (Dina *et al*. 2007b). In humans the highest *FTO* expression levels were found in the brain, then muscle, kidneys, adipose tissue, pancreas and liver (Frayling *et al*. 2007). Dina *et al*. (2007b) also reported human *FTO* expression in all these aforementioned tissues and additionally in the heart, placenta and lungs. In the brain the highest expression levels were found to be in the cerebral cortex, with high expression levels also reported for the parietal lobe, temporal lobe, hippocampus, cerebellum (Frayling *et al*. 2007), pituitary gland, hypothalamus and adrenal gland (Dina *et al*. 2007b, Frayling *et al*. 2007). Furthermore, in subcutaneous adipose tissue *FTO* expression was found to be higher in the adipocytes than in the rest of the adipose tissue (Wahlen *et al*. 2008). *FTO* was also found to be expressed in human fetuses with the highest levels in the fetal brain, then kidneys, liver and pancreas (Frayling *et al*. 2007).

Consistent with human data, *Fto* expression in mice was detected in all tissues tested (Peters *et al*. 1999, Gerken *et al*. 2007, Stratigopoulos *et al*. 2008) and found in mouse embryos (Gerken *et al*. 2007). In rodents, *Fto* was expressed in all 33 tissues tested including all brain slices encompassing the entire rat brain (Fredriksson *et al*. 2008). These studies also revealed highest *Fto* mRNA expression in the brain, specifically in the hypothalamus.

With *in situ* hybridization of hypothalamic slices it was shown that *Fto* mRNA was highly expressed in arcuate, paraventricular, dorsomedial and ventromedial nuclei of the mouse (Gerken *et al*. 2007) and rat hypothalamus (Fredriksson *et al*. 2008). Furthermore, *Fto* was found predominately in neurons and not in astrocytes or glia cells (Fredriksson *et al*. 2008).

Studies performed on humans, mice and rodents also indicated that *Fto* mRNA expression might be nutritionally regulated. In mice, fasting resulted in a 60% decreased *Fto* expression in the arcuate nucleus of the hypothalamus (Gerken *et al*. 2007). Similarly, Stratigopoulos *et al.* (2008) have shown decreased hypothalamic *Fto* expression in fasted obese and lean mice or in lean mice exposed to cold temperatures. However, contradictory to these results, rodents had an increased *Fto* expression in the hypothalamus following food deprivation (Fredriksson *et al*. 2008). Furthermore, monogenic obese mice had decreased *Fto* expression levels in mesenteric adipose tissue and several other tissues investigated compared to normal mice (Stratigopoulos *et al*. 2008). However, in humans *FTO* mRNA expression levels in subcutaneous adipose tissue of obese women (Wahlen *et al*. 2008) and morbidly obese adults (Zabena *et al*. 2009) were higher than the non-obese controls. Although these human, mice and rodent studies seem contradictory, all results point to the fact that nutritional intake, environmental conditions and weight status regulate *FTO* expression.

2.5.3 Physiological function of FTO

Recent research indicates that *FTO* probably encodes a 2-OG dependent nucleic acid demethylase. However, the physiological role of this protein in the development of obesity is still unclear (Gerken *et al*. 2007, Fischer *et al*. 2009). It is known that 2-OG dependent oxygenases are involved in DNA repair, fatty acid metabolism, and posttranslational modifications (Hausinger 2004, Clifton *et al*. 2006). *In vitro* experiments have shown that murine Fto catalyzes Fe(II) and 2-OG dependent demethylation of 3-methylthymine in single stranded DNA (ssDNA) (Gerken *et al*. 2007). Similarly, it was shown that mouse and human FTO are capable of repairing 3-methylthymine in ssDNA and 3-methyluracil in single stranded RNA (ssRNA) and prefer these substrates over other base lesions tested (Jia *et al*. 2008). However, no or negligible demethylation activities were observed for mouse and human FTO in double-stranded DNA and higher demethylation efficiency was also reported for ssRNA than ssDNA (Jia *et al*. 2008). These studies concluded that the FTO protein might exert gene regulation at RNA level in humans (Gerken *et al*. 2007, Jia *et al*. 2008). However, as a 2-OG dependent oxygenase it might also play a potential role in adaptation to hypoxia and lipolysis (Gerkin *et al*. 2007, Sanchez-Pulido & Andrade-Navarro 2007).

New insights into the functional role of *FTO* were recently provided by Fischer *et al*. (2009) who created Fto deficient homozygous ($Fto^{-/-}$) and heterozygous ($Fto^{-/+}$) mice and compared several measures of adiposity, dietary intake and energy expenditure between these mice and normal mice ($Fto^{+/+}$). No embryonic growth differences were observed between these mice, while the $Fto^{-/-}$ mice were growth retarded from day two after birth throughout their entire lifespan. These mice had a smaller length and after the age of six weeks they had a

generally 30 to 40% lower body weight. Furthermore, only the *Fto*$^{-/-}$ mice showed overall decreased measures of adiposity as they were characterized by a decreased fat mass, lean mass, adipocyte size, serum leptin, increased adiponectin and a complete loss of white adipose tissue (WAT) by the age of 15 months. However, both the *Fto*$^{-/-}$ and *Fto*$^{-/+}$ mice were protected from diet-induced obesity as their weight and WAT accumulation was lower compared to *Fto*$^{+/+}$ mice following a 12 week high fat diet. The general leanness associated with the *Fto*$^{-/-}$ mice was not related to a decreased food intake or major alterations of hypothalamic development but rather due to increased energy expenditure. The *Fto*$^{-/-}$ mice exibited higher day and night energy expenditure as measured by increased oxygen consumption, carbon dioxide production and calculated heat generation. This increase in energy expenditure occurred in the presence of lower physical activity levels and increased plasma epinephrine and thus increased sympathetic activation compared to the other mice. Further investigation into explanations for the increased energy expenditure in the presence of decreased physical activity indicated no involvement of mitochondrial uncoupling or altered thyroid function. This led the authors to suggest that altered futile cycling might possible explain these effects, however this hypothesis still needs to be confirmed. Because neuropeptide expressing neurons in the arcuate nucleus of the hypothalamus play an important role in the regulation of energy homeostasis, the expression of pro-opiomelanocortin-α (Pomc), agouti related peptide (AgRP) and neuropeptide Y (Npy) was measured during the fed and fasted state in the above mentioned mouse model. No differences between the mice were observed for the fed state, however during the fasted state *Fto*$^{-/-}$ mice had decreased Pomc and Npy levels (Fischer *et al*. 2009).

One study on human adipocytes indicated that increased expression of *FTO* mRNA is related to increased leptin and perilipin but not adiponectin gene expression in subcutaneous adipose tissue, as well as with increased perilipin expression in visceral adipose tissue (Zabena *et al*. 2009).

The fact that high levels of *FTO* expression are found in the brain, specifically in the arcuate nucleus of the hypothalamus, which is known to play a major role in controlling energy homeostasis and eating behaviour, together with new insights from recent *in vitro* studies indicating a possible role in energy expenditure, provides support for a role of *FTO* in the explanation of obesity development.

2.5.4 The *FTO* rs1421085 and rs17817449 polymorphisms

The first *FTO* polymorphisms associated with obesity were identified through a genome wide association (GWA) study searching for novel genes associated with type-2 diabetes in diabetic cases and population controls from the Wellcome Trust Case Control Consortium in the United Kingdom (Frayling *et al*. 2007). From scanning 490032 autosomal polymorphisms in various genes these authors revealed that the strongest association between a polymorphism and type-2 diabetes was found for the rs9939609 polymorphism in the *FTO* gene. A strong association was also found between this *FTO* polymorphism and BMI and it was subsequently shown that the association with diabetes disappears with adjustment for BMI. Frayling *et al*. (2007) have also shown strong association signals with BMI in a cluster of ten polymorphisms found in the first intron of *FTO*, of which

rs9939609 polymorphism forms part. These results were soon confirmed with three other GWA studies carried out on obese class III French Caucasians (Dina *et al*. 2007b), obese and lean German children and Sardinians (Scuteri *et al*. 2007). From these initial GWA studies several *FTO* polymorphisms were identified and further tested in large cohort, case-control and family studies all showing strong and consistent associations with BMI and obesity in European Caucasians (Dina *et al*. 2007b, Frayling *et al*. 2007, Scuteri *et al*. 2007). The polymorphisms are all located within a high LD block spanning a 47 kb region and includes not only intron 1 but also exon 2 of *FTO* (Dina *et al*. 2007b, Frayling *et al*. 2007, Scuteri *et al*. 2007). The polymorphisms showing the strongest association with obesity, include the rs9939609 (Frayling *et al*. 2007), rs9930506 (Scuteri *et al*. 2007), rs1421085, rs17817449 and rs1121980 variants (Dina *et al*. 2007b). The mutant alleles of these polymorphisms were consistently found to be associated with a higher BMI or with obesity at significant levels unobserved for the contribution of genetics to common obesity (Dina *et al*. 2007b). These studies indicate that the *FTO* polymorphisms yield a population-attributable risk of 22% (Dina *et al*. 2007b) and 20% (Frayling *et al*. 2007) for common obesity in Caucasian European populations. However, thus far no association with obesity for an exonic *FTO* polymorphisms has been found and because of the strong LD between the various intronic polymorphisms, the functional polymorphism of *FTO* has not yet been determined (Dina *et al*. 2007b).

For the purposes of this study the *FTO* rs1421085 and rs17817449 polymorphisms were investigated and therefore only these two polymorphisms are discussed further. The *FTO* rs1421085 polymorphism results in a T to C transition, while the *FTO* rs17817449 polymorphism results in a T to G transition. Both polymorphisms are located within intron 1 of the *FTO* gene (Dina *et al*. 2007b). It is unknown whether these polymorphisms influence the expression and regulation of the FTO protein (Fischer *et al*. 2009). When considering studies investigating other polymorphisms in the same *FTO* locus it was found that the *FTO* rs9939609 polymorphism had no effect on *FTO* mRNA expression levels (Wahlen *et al*. 2008) or on the expression of genes regulating lipolysis such as leptin, perilipin and HSL in subcutaneous and visceral adipose tissue (Wahlen *et al*. 2008, Zabena *et al*. 2009). However, healthy women who are homozygous for the wild-type *FTO* rs9939609 genotype seem to be protected from obesity development due to their 28% higher *in vivo* lipolytic activity compared to the mutant allele carriers (Wahlen *et al*. 2008). Furthermore, these women also had 22% higher *in vitro* basal lipolysis levels as measured by adipocyte glycerol release (Wahlen *et al*. 2008).

2.5.5 Genotype and allele frequencies

In Caucasian populations with European ancestry the frequency for the wild-type *FTO* rs1421085 TT genotype ranges between 23 to 38% (Table 2.16). In obese groups, the frequency of the TT genotype was more towards the lower end of this range (23 to 27%) compared to frequencies from population-based samples or non-obese controls that were more towards the upper range of 34 to 38%. The frequency of the mutant CC genotype ranges between 11 and 39%, with the frequency in the obese more towards the upper end of this range (20 to 28%) and population-based samples or controls between 11 and 22%. The genotype frequencies of only one Asian population have been published and are similar to those of Hispanic-Americans. When compared to

Caucasian populations the CC genotype frequencies were lower (4 to 5%) and the TT genotype frequencies were higher (58 and 67%) in the Asian and Hispanic-American populations. The C-allele frequency ranges between 0.37 and 0.59 for Caucasians, which is higher than the frequencies of 0.04 to 0.35 reported for Asian and Oceanic populations as well as for Hispanic and African Americans.

Table 2.16: Genotype frequency of the *FTO* rs1421085 polymorphism and frequency of the mutant C-allele.

Population	Weight or disease status	n	TT %	TC %	CC %	C-allele	References
Caucasian							
Romanian ♀	All PCOS	207	26	49	25	52	Attaoua *et al*. 2008
	Control lean	100			16	46	
	PCOS lean	107			22	46	
	PCOS obese	100			28	52	
	MetS in PCOS	75			39	59	
Finnish children		5532	35	48	17	41	Cauchi *et al*. 2009
French		4726	34	49	17	42	Cauchi *et al*. 2009
French-Canadian		359	38	44	18	41	Do *et al*. 2008
French children & adults	Obese and non-obese					49	Stutzmann *et al*. 2009
French	Obese class III	867	23	49	28	52	Dina *et al*. 2007b
	Non-obese	2411	35	47	18	41	
Swiss	Obese class III					51	Stutzmann *et al*. 2009
Non-Hispanic Americans	Obese cases	523	26	47	27	51	Price *et al*. 2008
	Obese sisters	93	25	55	20	48	
	Thin sisters	92	34	55	11	39	
	Controls	521	37	52	11	37	
Belgian Caucasians	Controls	268	35	47	18	41	Peeters *et al*. 2008
	Obese cases	1099	27	50	23	48	
Hispanic American							
Hispanic Americans		1237	61	34	5	20	Wing *et al*. 2009
Africa							
African Americans		581				14	Wing *et al*. 2009
Asian							
Japanese	Obese	924	58	37	5	24	Hotta *et al*. 2008b
	Control	1519	67	29	4	18	
Singapore: Chinese		2919				12	Tan *et al*. 2008
Singapore: Malay		785				28	
Singapore: Malay		2996				29	
Singapore: Indian		594				35	
Oceanic populations							
Munda		39				24	Ohashi *et al*. 2008
Paradise		96				11	
Rawaki		96				4	
Balopa		39				13	
Gidra		48				11	
Tongan		198				17	
Thai		48				18	

♀ = women, PCOS = poly-cystic ovary syndrome, Obese class III = BMI≥40 kg/m^2

For the *FTO* rs17817449 polymorphism, the GG genotype ranges between 26 and 40% for Caucasians, while the TT genotype frequency ranges between 8 and 27% (Table 2.17). Again the genotype frequencies reported in the different studies for obese subjects were similar, but different from frequencies reported for non-obese subjects. The genotype frequencies for populations with Asian ancestry were not published by Ohashi *et al*. (2008) or Tan

et al. (2008), but it can be speculated that it may be similar to those of Hispanic Americans as was found for the *FTO* rs1421085 polymorphism.

The G-allele frequency ranges between 0.48 and 0.51 for obese Caucasians and 0.36 to 0.40 for non-obese or population samples. The G-allele frequency for Hispanic, Asian and Oceanic populations is lower and ranges between 0.05 and 0.34.

Table 2.17: Genotype frequency of the *FTO* rs17817449 polymorphism and frequency of the mutant G-allele.

Population	Weight and disease status	n	TT %	TG %	GG %	G-allele %	References
Caucasian							
French Canadian		359	18	42	40	39	Do *et al*. 2008
French	Obese class III	873	27	49	24	51	Dina *et al*. 2007b
	Non-obese	2400	16	48	36	40	
Non-Hispanic	Controls	522	11	50	39	36	Price *et al*. 2008
Caucasians	Obese cases	527	26	47	28	49	
	Thin sisters	98	8	57	35	37	
	Obese sisters	93	23	52	26	48	
Hispanic American							
Hispanic Americans		1235	6	35	59	22	Wing *et al*. 2009
Africa							
African Americans		581				46	Wing *et al*. 2009
Asian							
Singapore: Chinese		2919				12	Tan *et al*. 2008
Singapore: Malay		785				28	
Singapore: Malay		2996				30	
Singapore: Indian		594				34	
Oceanic populations							
Munda		39				30	Ohashi *et al*. 2008
Paradise		96				19	
Rawaki		96				5	
Balopa		39				13	
Gidra		48				10	
Tongan		198				18	
Thai		48				18	

Obese class III = BMI ≥ 40 kg/m^2

2.5.6 Associations with obesity related phenotypes

Weight, BMI and obesity

Consistent evidence from small and large-scale studies shows that the *FTO* rs1421085 polymorphism is associated with obesity and BMI in Caucasian populations with European ancestry (Table 2.18) (Dina *et al*. 2007b, Attaoua *et al*. 2008, Do *et al*. 2008, Peeters *et al*. 2008, Price *et al*. 2008, Cauchi *et al*. 2009, Stutzmann *et al*. 2009). Most of these studies compared the BMI of subjects or the genotype frequencies of obese cases with controls using the additive model (CC vs. CT vs. TT). It was evident that the CC homozygotes had significantly higher BMIs or frequency in obese cases than the CT heterozygotes, while these measures for the CT

heterozygotes were again significantly higher than for the TT homozygotes. The CC or CT genotypes were associated with higher BMIs from birth through childhood, adolescence and adulthood, while it was associated with obesity in children from as young as seven years (Cauchi *et al*. 2009). The association with obesity in children was consistently shown in Finnish (Cauchi *et al*. 2009), French and German children (Dina *et al*. 2007b). In family based studies using pedigree analysis it was also observed that the C-allele is over-transmitted to obese French children (Dina *et al*. 2007b, Stutzmann *et al*. 2009) and French and Swedish severely obese adults (Dina *et al*. 2007b). In patients with polycystic ovary syndrome (PCOS) it was found that the risk for obesity was higher in those who had the CC homozygous genotype (Attaoua *et al*. 2008). Even in lean PCOS patients, having the CC genotype resulted in a significantly higher BMI and therefore these patients have a higher risk of becoming obese when compared to TT homozygous PCOS patients (Attaoua *et al*. 2008).

The two studies thus far that investigated the role of the *FTO* rs1421085 polymorphism in African-Americans, found no association with BMI (Scuteri *et al*. 2007, Wing *et al*. 2009). These authors speculate that the lower reported C-allele frequency (see Table 2.16) and the lower LD found between the *FTO* variants in individuals of African descent might explain this lack of association with BMI and obesity (Dina *et al*. 2007b, Scuteri *et al*. 2007, Wing *et al*. 2009). The one study conducted on Asian populations indicated that a higher BMI was associated with the CC and CT genotypes in Malayan and Chinese subjects living in Singapore. However, no association was found for Indian subjects living in Singapore (Tan *et al*. 2008).

The *FTO* rs17817449 polymorphism shows similar associations with BMI and obesity as discussed for the *FTO* rs1421085 polymorphism (Table 2.19). The GG and GT genotypes or the mutant G-allele of the *FTO* rs17817449 polymorphism have consistently been found to be associated with a higher BMI or higher obesity prevalence in Caucasians with European ancestry (Dina *et al*. 2007b, Do *et al*. 2008, Price *et al*. 2008). The G-allele was also over-transmitted to French obese children and French and Swedish obese adults (Dina *et al*. 2007b). These associations were not found for subjects with African ancestry, more specifically for Gambians (Hennig *et al*. 2009) and African-Americans (Wing *et al*. 2009). In Asian populations a higher BMI was also found to be associated with the GG and GT genotypes in Malayan and Chinese subjects living in Singapore, with no association found for Indians from Singapore (Tan *et al*. 2008).

In summary, these findings indicate that the C-allele of the *FTO* rs1421085 polymorphism and the G-allele of the *FTO* rs17817449 polymorphism are strongly and consistently associated with a higher BMI and with early-onset obesity in Caucasians. This is in agreement with the findings from several other studies reporting significant associations between BMI and/ or obesity in Caucasians and the mutant alleles of other polymorphisms in strong LD with the *FTO* rs17817449 and rs1421085 polymorphisms also found in intron 1 of the *FTO* gene (Cornes *et al*. 2009, Frayling *et al*. 2007, Freathy *et al*. 2008, Grant *et al*. 2008, Hunt *et al*. 2008, Kring *et al*. 2008, Qi *et al*. 2008, Rampersaud *et al*. 2008). However, current evidence points to an absence of such associations with the *FTO* rs1421085 or rs17817449 polymorphisms in populations with African ancestry. More research is needed to draw final conclusions regarding such associations in populations with Asian ancestry.

Table 2.18: The association between the *FTO* rs1421085 polymorphism and weight, BMI and obesity.

Population	Weight or disease status	n	Study design	Association with Obesity		Association with BMI		Reference
				Yes	No	Yes	No	
Caucasian								
Americans:	Controls Obese cases	521 523	Case-control	Obese = ↑ C-allele	-	ni	ni	Price *et al*. 2008
Americans:	Thin sisters Obese sisters	92 93	Case-control	-	✓	ni	ni	Price *et al*. 2008
Americans:		1496	Family based	ni	ni	C-allele = ↑ BMI	-	Scuteri *et al*. 2007
Romania ♂	PCOS obese PCOS lean Lean controls	100 107 100	Case-control: unrelated ♂ with PCOS and lean controls. PCOS divided in obese and lean groups	Obese = ↑ CC	-	ni	ni	Attaoua *et al*. 2008
Romania ♂	All with PCOS	207	as above	ni	ni	-	✓	Attaoua *et al*. 2008
Romania ♂	Lean PCOS	107	as above	ni	ni	CC = ↑ BMI	-	Attaoua *et al*. 2008
French Canadian		908	Family-based	ni	ni	CC = ↑ BMI CC/CT = ↑ BMI	-	Do *et al*. 2008
French		4726	Prospective cohort	-	✓	CC/CT = ↑ BMI	-	Cauchi *et al*. 2009
Finnish children		5532	Prospective cohort: at birth 7 years 16 years	- Obesity = ↑ CC/CT Obesity = ↑ CC/CT	✓ - -	CC/CT = ↑ BMI CC/CT = ↑ BMI CC/CT = ↑ BMI	-	Cauchi *et al*. 2009
French: children adults	Obese Non-obese Obese Non-obese	1004 313 2438 2009	Family study with pedigree analysis including children, parents and grandparents	C-allele = overtransmitted to obese subjects	-	C-allele = ↑ BMI z-score	-	Stutzmann *et al*. 2009
French adults:	obese class III non-obese	867 2411	Case-control	Obese = ↑ CC/CT	-	ni	ni	Dina *et al*. 2007b
French: children adults	obese non-obese	702 979	Case-control	Obese = ↑ CC/CT	-	ni	ni	Dina *et al*. 2007b
French: children adults	obese non-obese	482 519	Case-control	Obese = ↑ CC/CT	-	ni	ni	Dina *et al*. 2007b
French: children adults	obese obese class III		Family-based: obese parents and sibs	C-allele transmitted to obese children & adults	-	ni	ni	Dina *et al*. 2007b
Swedish: adults	obese class III	154	Family-based: discordant for obesity class III	C-allele transmitted to obese	-	ni	ni	Dina *et al*. 2007b
German: children	obese non-obese	283 699	Case-control	Obese = ↑ CC/CT	-	ni	ni	Dina *et al*. 2007b
Belgium :	Obese	1099	Case-control	Obese = ↑ CC/CT	-	CC+CT = ↑ BMI	-	Peeters *et al*. 2008

Table 2.18: *continued*

Population	Weight or disease status	n	Study design	Association with Obesity		Association with BMI		Reference
				Yes	No	Yes	No	
Caucasian								
Swiss:	obese class III	504	Case-control	Obese = ↑ CC/CT	-	ni	ni	Dina *et al*. 2007b
	all	514						
Swiss	obese class III	1274	Purpositively selected	ni	ni	-	✓	Stutzmann *et al*. 2009
Swiss and French		7038	Case-control	Obese = ↑ C-allele	-	ni	ni	Stutzmann *et al*. 2009
Hispanic American								
Hispanic Americans		839	Family-based	ni	ni	-	✓	Scuteri *et al*. 2007
Hispanic Americans		1237	Family-based	ni	ni	CC/CT = ↑ BMI	-	Wing *et al*. 2009
African								
African Americans		1101	Family-based	ni	ni	-	✓	Scuteri *et al*. 2007
African Americans		604	Family-based	ni	ni	-	✓	Wing *et al*. 2009
Asian								
Japanese	Obese	924	Case-control	Obese = ↑ CC/CT		CC/CT = ↑ BMI	-	Hotta *et al*. 2008b
	Controls	1519		Obese = ↑ CC		CC+CT = ↑ BMI		
Singapore: Malay		2996	Cross-sectional	ni	ni	CC/CT = ↑ BMI	-	Tan *et al*. 2008
Singapore: Malay		785		ni	ni	CC/CT = ↑ BMI	-	
Singapore: Indian		594		ni	ni	-	✓	
Singapore: Chinese		2919		ni	ni	CC/CT = ↑ BMI	-	

ni = not investigated, Mutant allele = C-allele, Genotype frequency comparisons: CC+CT = Dominant model (TT vs. CC+CT), CC = recessive model (CC vs. CT+TT), CC/CT = additive model (CC vs. CT vs. TT), Allele frequency comparisons: C-allele = compared vs. G-allele

Table 2.19: The association between the *FTO* rs17817449 polymorphism and weight, BMI and obesity.

Population	Weight or disease status	n	Study design	Association with obesity		Association with BMI		Reference
				Yes	No	Yes	No	
Caucasian								
Americans:	Controls	521	Case-control	Obese = ↑ G-allele	-	ni	ni	Price *et al*. 2008
	Obese cases	523						
Americans:	Lean sisters	92	Case-control	Obese = ↑ G-allele	-	ni	ni	Price *et al*. 2008
	Obese sisters	93						
French Canadian		908	Family-based	ni	ni	GG = ↑ BMI	-	Do *et al*. 2008
						GG/GT = ↑ BMI		

Table 2.19: *continued*

Population	Weight or disease status	n	Study design	Association with obesity		Association with BMI		Reference
				Yes	No	Yes	No	
Caucasian								
French adults:	obese class III non-obese	873 2400	Case-control	Obese = ↑ GG/GT	-	ni	ni	Dina *et al*. 2007b
French: children adults	obese non-obese	693 990	Case-control	Obese = ↑ GG/GT	-	ni	ni	Dina *et al*. 2007b
French: children young adults	obese non-obese	485 525	Case-control	Obese = ↑ GG/GT	-	ni	ni	Dina *et al*. 2007b
French: children adults	obese obese class III	685 111	Family-based: obese parents and sibs	G-allele transmitted to obese children & adults	-	ni	ni	Dina *et al*. 2007b
Swedish: adults	obese class III	154	Family-based: discordant for obesity class III	G-allele transmitted to obese	-	ni	ni	Dina *et al*. 2007b
German:	obese non-obese	281 691	Case-control	Obese = ↑ GG/GT	-	ni	ni	Dina *et al*. 2007b
Swiss:	obese class III all	516 519	Case-control	Obese = ↑ GG/GT	-	ni	ni	Dina *et al*. 2007b
French, Swiss, Germans	Obese non-obese	2848 5125	Meta-analysis of all case-control samples	Obese = ↑ GG/GT	-	ni	ni	Dina *et al*. 2007b
Hispanic American								
Hispanic Americans		1235	Family-based	ni	ni	GG/GT = ↑ BMI	-	Wing *et al*. 2009
African								
Gambians		2164	All children of moms enrolled in antenatal scheme	ni	ni	-	✓	Hennig *et al*. 2009
African Americans		604	Family-based	ni	ni	-	✓	Wing *et al*. 2009
Asian								
Singapore: Malay		2996	Population based (SiMES)	ni	ni	GG/GT = ↑ BMI	-	Tan *et al*. 2008
Singapore: Malay		785	Population based (NHS98)	ni	ni	GG/GT = ↑ BMI	-	
Singapore: Indian		594	Population based	ni	ni	-	✓	
Singapore: Chinese		2919	Population based	ni	ni	GG/GT = ↑ BMI	-	

ni = not investigated, mutant allele = G-allele, Genotype frequency comparisons: GG+GT = Dominant model (TT vs. GG+GT), GG = recessive model (GG vs. GT+TT), GG/GT = additive model (CC vs. CG vs. GG), Allele frequency comparisons: G-allele = compared vs. T-allele, BMI = body mass index, SiMES = Singapore Malay Eye Study, NHS98 = 1998 Singapore National Health Survey.

Measures of body fat distribution and content

The mutant C-allele of the *FTO* rs1421085 polymorphism has been associated with a higher waist circumference, hip circumference, waist-hip ratio, body fat percentage, sum of six skinfolds, fat mass, total fat area, subcutaneous fat area and subcutaneous adipose tissue in Caucasian populations (Table 2.20). The CC and CT genotypes were also associated with a higher waist circumference in Asian populations, however no association was found in African-Americans.

Table 2.20: The association between the *FTO* rs1421085 polymorphism and measures of body fat distribution and content.

Population	Associations	No associations	Reference
Caucasian			
Belgian	CC+CT = ↑ fat mass, TFA, SFA	VFA, FFM, HC, WC, WHR	Peeters *et al*. 2008
Romanian ♀ with PCOS	-	WC	Attaoua *et al*. 2008
French Canadian	CC = ↑ FM, FFM, WC, HC, WHR, % body fat, sum of 6 skinfolds	-	Do *et al*. 2008
French	CC/CT = ↑ fat mass	-	Cauchi *et al*. 2009
Hispanic American			
Hispanic American	CC/CT = ↑ WC, SAT	WHR, VAT, VSR	Wing *et al*. 2009
African			
African-American	-	WC, WHR, VAT, SAT, VSR	Wing *et al*. 2009
Asian			
Singapore: Malay (NHS98)	CC/CT = ↑ WC	WHR	Tan *et al*. 2008
Singapore: Indian	-	WC, WHR	
Singapore: Chinese	CC/CT = ↑ WC	WHR	

VFA = visceral fat area, SFA = subcutaneous fat area, TFA = total fat area, PCOS = polycystic ovary syndrome, FM = fat mass, FFM = fat free mass, HC = hip circumference, WC = waist circumference, WHR = waist-hip-ratio, SAT = subcutaneous adipose tissue, VAT = visceral adipose tissue, VSR = visceral to subcutaneous ratio.

Only three studies investigated the association between measures of body fat distribution and fat content with the *FTO* rs17817449 polymorphism (Table 2.21). The mutant G-allele, either in the form of homozygotes or with an additive model was associated with a higher fat mass, waist circumference, hip circumference, waist-hip ratio, body fat percentage, sum of six skinfolds and subcutaneous adipose tissue in Caucasian populations. The GG and GT genotypes were also associated with a higher waist circumference in Asian populations, however no association was found in African-Americans.

Table 2.21: The association between the *FTO* rs17817449 polymorphism and measures of body fat distribution and content.

Population	Associations	No associations	Reference
Caucasian			
French Canadian	GG = ↑ FM, FFM, WC, HC, WHR, % body fat, sum of 6 skinfolds	-	Do *et al*. 2008
Hispanic American			
Hispanic Americans	GG/GT = ↑ SAT	WC, WHR, VAT, VSR	Wing *et al*. 2009
African			
African-American	-	WC, WHR, VAT, SAT, VSR	Wing *et al*. 2009
Asian			
Singapore: Malay (NHS98)	GG/GT = ↑ WC	WHR	Tan *et al*. 2008
Singapore: Indian	-	WC, WHR	
Singapore: Chinese	GG/GT = ↑ WC	WHR	

FM = fat mass, FFM = fat free mass, HC = hip circumference, WC = waist circumference, WHR = waist-hip-ratio, SAT = subcutaneous adipose tissue, VAT = visceral adipose tissue, VSR = visceral to subcutaneous ratio.

2.5.7 Associations with health indicators

Indicators of glucose and insulin homeostasis

In general, the C-allele or the CC and CT genotypes of the *FTO* rs1421085 polymorphism were associated with higher plasma glucose levels as well as measures indicating insulin resistance (Table 2.22). French CC homozygous subjects were more insulin resistant - as indicated by a lower metabolic clearance rate and insulin sensitivity index following an oral glucose tolerance test - than T-allele carriers (Do *et al*. 2008). The prevalence and nine year incidence of Type 2 diabetes was also found to be higher in CC homozygous French subjects. However, it is likely that this effect was mediated through BMI as the association disappeared after adjustment for BMI (Cauchi *et al*. 2009). Romanian CC homozygous women with PCOS had higher fasting glucose, two-hour glucose and AUC for glucose as well as a higher prevalence of impaired glucose tolerance (IGT), impaired fasting glucose (IFG) and the MetS (NCEP ATP III criteria) (Attaoua *et al*. 2008). All components of MetS were more prevalent in C-allele carriers, but the most significant was fasting hyperglycemia. Even lean CC homozygous PCOS patients were more insulin resistant according to the HOMA-IR test, had higher fasting glucose levels and a higher prevalence for IFG and MetS (Attaoua *et al*. 2008). No association was found for African-Americans and Hispanic-Americans (Wing *et al*. 2009). The one study conducted on Asian populations indicated higher fasting glucose levels associated with the C-allele according to the additive model (Tan *et al*. 2008)

Similar patterns of associations as reported for the *FTO* rs1421085 polymorphism were found for measures of glucose metabolism and insulin resistance and the G-allele according to the additive model or the GG genotype of the *FTO* rs17817449 polymorphism in the different population groups (Table 2.23).

In summary, available research results indicate that the mutant C-allele of the *FTO* rs1421085 polymorphism and G-allele of the *FTO* rs17817449 polymorphism might be associated with impaired glucose homeostasis, insulin resistance and consequently the development of Type-2 diabetes in Caucasians. However, more research is necessary to confirm these associations in all ancestral groups.

Blood lipid profile and blood pressure

No studies could be found that investigated the association of the *FTO* rs1421085 and rs17817449 polymorphisms and plasma lipid profile in Caucasians or African-Americans (Tables 2.22 and 2.23). Only Tan *et al*. (2008) reported an association between the mutant alleles of these two polymorphisms and higher plasma LDL cholesterol in Chinese living in Singapore. The C-allele of the *FTO* rs1421085 polymorphism was also associated with higher plasma triglycerides in Malayans living in Singapore included in the 1998 National Health Survey (NHS98). However this association was not found in Malayans participating in the Singapore Malay Eye Study (SiMES) (Tan *et al*. 2008).

In summary, more research is necessary to elucidate the associations between the *FTO* rs1421085 and rs17817449 polymorphisms and plasma lipid profile.

2.5.8 Associations with weight loss outcomes

No reports could be traced regarding weight loss interventions and association with the *FTO* rs1421085 or rs17817449 polymorphisms. However, two reports that investigated the role of the *FTO* rs9939609 polymorphism (also found in intron 1 of the *FTO* gene and in strong LD with the *FTO* rs1421085 and rs17817449 polymorphisms) and weight loss following weight loss interventions, were found. These studies showed no association between the *FTO* rs9939609 polymorphism and weight loss outcome at 10 to 12 months in obese German children (n=207) following the "Obeldicks" intervention programme (Müller *et al*. 2008) or in overweight non-diabetic American adults (n=3533) who were treated with either a placebo, or metformin or troglitazone or a lifestyle intervention in the Diabetes Prevention Programme (DPP) (Franks *et al*. 2008). The "Obeldicks" (Reinehr *et al*. 2008, Reinehr *et al*. 2009) and DPP (Franks *et al*. 2008) intervention programmes are summarized in Table 2.15, p70.

The work by Stutzmann *et al*. (2009) on the association between eating behaviour of children and adults with European ancestry and the *FTO* rs1421085 polymorphism should also be considered in this regard. In French obese and non-obese children no association between the *FTO* rs1421085 polymorphism and eating large quantities of food, frequent snacking or cravings was found. No association was found with snakcing during weekends or eating large quantities of food at meals in 16 year olds who participated in a Finnish Birth cohort. Furthermore, in adults no association between the *FTO* rs1421085 polymorphism and scores on the three factor eating questionnaire was found in obese and non-obese French adults, or with snacking and eating large quantities of food at meals in Swiss class III obese adults or with snacking and bulimia in French adults from a randomly selected population study (Stutzmann *et al*. 2009). These cross-sectional analyses of children and adults with different weight classifications indicate that no association between the eating behaviour and the *FTO* rs1421085 polymorphism seems to exist.

Table 2.22: The association between the *FTO* rs1421085 polymorphism and indicators of glucose and insulin homeostasis, blood lipid profile and blood pressure.

Population	Indicators of glucose and insulin homeostasis		Blood lipids		Reference
	Association	No association	Association	No associations	
Caucasian					
French Canadian	CC = ↓ MCR-OGTT, ↓ ISI-OGTT	insulin, insulin AUC, glucose, AUC for glucose, HOMA-IR	CC = ↑ leptin	-	Do *et al*. 2008
French	CC = ↑ DM2 prevalence and incidence over 9 yrs (not after adjustment for BMI)	-	ni	ni	Cauchi *et al*. 2009
Romanian ♀	*All PCOS:* CC = ↑ glucose, 2h glucose, AUC for glucose, IGT, IFG, MetS *Lean controls:* CC = ↑ IGT *Lean PCOS:* CC = ↑ glucose, IFG, HOMA-IR, MetS *PCOS with MetS:* CC = ↑ 2h glucose, 2h insulin, AUC for glucose, HOMA-IR, vs. obese PCOS	All parameters were measured in all groups, therefore those not indicated under association column = no association in that specific group	ni	ni	Attaoua *et al*. 2008
Hispanic American					
Hispanic Americans	-	Glucose, insulin, ISI, AIR, DI	ni	ni	Wing *et al*. 2009
African					
African-Americans	-	Glucose, insulin, ISI, AIR, DI	ni	ni	Wing *et al*. 2009
Asian					
Singapore: Malay (SiMES)	CC/CT = ↑ glucose	IFG/IGT	-	LDL, HDL, TG, BP	Tan *et al*. 2008
Singapore: Malay (NHS98)	CC/CT = ↑ glucose	IFG/IGT	CC/CT = ↑ TG	LDL, HDL, BP	
Singapore: Indian	-	glucose, IFG/IGT	-	LDL, HDL, TG, BP	
Singapore: Chinese	CC/CT = ↑ glucose	IFG/IGT	CC/CT = ↑ LDL	HDL, TG, BP	

ni = not investigated, PCOS = polycystic ovary syndrome, AUC = area under the curve, IFG = impaired fasting glucose, IGT = impaired glucose tolerance, HOMA-IR = Homeostasis model assessment of insulin resistance, MetS = Metabolic Syndrome, MCR-OGTT: Metabolic Clearance Rate derived from the Oral Glucose Tolerance Test; ISI-OGTT: Insulin Sensitivity Index derived from the Oral Glucose Tolerance Test, DM2 = Type 2 diabetes, AIR = acute insulin response, DI = deposition index, TG = triglycerides, TC = Total Cholesterol, HDL = High density lipoprotein, LDL = low density lipoprotein, BP = blood pressure.

Table 2.23: The association between the *FTO* rs17817449 polymorphism and indicators of glucose and insulin homeostasis, blood lipid profile and blood pressure.

Population	Glucose and insulin homeostasis		Blood lipids and blood pressure		Reference
	Association	No association	Association	No associations	
Caucasian					
French Canadian	GG = ↑ insulin, insulin AUC, ↓ MCR-OGTT	glucose, AUC for glucose, HOMA-IR, ISI-OGTT	GG = ↑ leptin	-	Do *et al*. 2008
Hispanic American					
Hispanic-Americans	-	Glucose, insulin, ISI, AIR, DI	ni	ni	Wing *et al*. 2009
African					
African-Americans	-	Glucose, insulin, ISI, AIR, DI	ni	ni	Wing *et al*. 2009
Asian					
Singapore: Malay (SiMES)	GG/GT = ↑ glucose	IFG/IGT	-	LDL, HDL, TG, BP	Tan *et al*. 2008
Singapore: Malay (NHS98)	GG/GT = ↑ glucose	IFG/IGT	-	LDL, HDL, TG, BP	
Singapore: Indian	-	glucose, IFG/IGT	-	LDL, HDL, TG, BP	
Singapore: Chinese	GG/GT = ↑ glucose	IFG/IGT	GG/GT = ↑ LDL	HDL, TG, BP	

ni = not investigated, AUC = area under the curve, IFG = impaired fasting glucose, IGT = impaired glucose tolerance, HOMA-IR = Homeostasis model assessment of insulin resistance, MCR-OGTT = Metabolic Clearance Rate derived from the Oral Glucose Tolerance Test, ISI-OGTT = Insulin Sensitivity Index derived from the Oral Glucose Tolerance Test, AIR = acute insulin response, DI = deposition index, TG = triglycerides, TC = Total Cholesterol, HDL = High density lipoprotein, LDL = low density lipoprotein, BP = blood pressure.

2.6 The β_3-Adrenergic receptor gene (β_3-AR and *ADRB3*; β_3-adrenoceptor)

2.6.1 The *ADRB3* gene

The human β_3-adrenergic receptor gene (*ADRB3*) was first cloned in 1989 (Emorine *et al*. 1989) and is located on chromosome position 8p12-p11.2 (Wilkie *et al*. 1993). The *ADRB3* gene consists of 3699 bp and has two exons separated by one intron (Rozec & Gauthier 2006, NCBI 2009).

2.6.2 The ADRB3 protein

The *ADRB3* gene encodes a 408 amino acids protein, namely the β_3-adrenergic receptor. The first exon of *ADRB3* encodes for the first 402 amino acids of the receptor and the second exon encodes the last six amino acids of the C-terminus tail and the 3' region not translated from the mRNA (Rozec & Gauthier 2006, NCBI 2009).

The β_3-adrenergic receptor is a member of the family of adrenergic receptors (also referred to as adrenoceptors) comprising of the α-adrenoceptors and β-adrenoceptors. The α-adrenoceptors are divided into six subtypes, namely the α_{1A}-, α_{1B}-, α_{1D}-, α_{2A}-, α_{2B}-, α_{2C}-adrenoceptors, while the β-adrenoceptors are divided into three subtypes namely the β_1-adrenoceptors (*ADRB1* or β_1AR), β_2-adrenoceptors (*ADRB2* or β_2AR) and β_3-adrenoceptors (ADRB3 or β_3AR) (Emorine *et al*. 1989, Small *et al*. 2003, Robidoux *et al*. 2004).

All adrenoceptors are members of the Guanine binding protein coupled receptors (GPCRs). The GPCRs are cell surface receptors and thus integral proteins of plasma membranes (Small *et al*. 2003, Robidoux *et al*. 2004). The general structure of all GPCRs, including the β-adrenoceptors is characterized by seven transmembrane domains of 22–28 amino acids each with three intracellular and three extracellular loops (Figure 2.4). The amino (NH_2) terminus of all three β-adrenoceptors is glycosylated and located extracellular, whereas the carboxy (COOH) terminus is found intracellular (Small *et al*. 2003, McGraw & Liggett 2005, Rozec & Gauthier 2006). A 65 to 70% amino acid sequence homology exists between the β1-, β2- and β3-adrenoceptors in humans, mostly in the seven transmembrane segments and the membrane-proximal regions of the intracellular loops (Small *et al*. 2003, Johnson 2006, Rozec & Gauthier 2006). Figure 2.4 also illustrates the position on the GPCRs where important single nucleotide polymorphisms with clinical relevance of seven of the nine adrenergic genes have been found (Small *et al*. 2003)

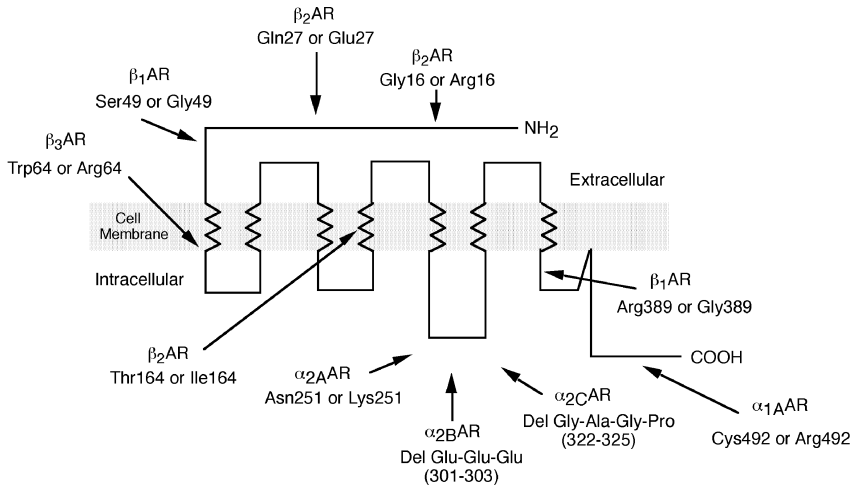

Figure 2.4: General structure of the human adrenoceptors and the approximate location of polymorphisms of human adrenergic receptors (from Small *et al*. 2003).

In humans, *ADRB3* is mainly expressed in WAT and BAT, especially in the fat and adipocytes lining the gastrointestinal tract, thus mainly in visceral fat tissue (Emorine *et al*. 1989, Krief *et al*. 1993, Rohrer 1998, Lowell & Bachman 2003, Robidoux *et al*. 2004). *ADRB3* is also expressed in various other human tissues (Small *et al*. 2003).

2.6.3 Physiological function of ADRB3

ADRB3 is primarily involved in the regulation of energy metabolism through influencing resting metabolic rate (thus energy expenditure), thermogenesis and lipolysis (Emorine *et al*. 1989, Loktionov 2003, Loos & Bouchard 2003) on activation of the sympathetic nervous system (Lofantan & Berlan 1993, Small *et al*. 2003). Activation of ADRB3 in white fat cells results in the mobilization of lipids (lipolysis) and in brown fat cells thermogenesis is increased (Loktionov 2003, Small *et al*. 2003). In general, the β-adrenoceptors stimulate fat lipolysis while the α-adrenoceptors inhibit fat lipolysis (Robidoux *et al*. 2004).

The GPCRs, including ADRB3, represent the largest signalling family in the human genome and transduce signals from the outside to the inside of cells from various host systems including sight, smell, hormonal, neurotransmitter, autocrine, and paracrine systems (McGraw & Liggett 2005). GPCR cell signalling occurs through coupling with the Guanine nucleotide binding proteins (G-proteins) (Robidoux *et al*. 2004, McGraw & Liggett 2005). Several G-proteins exist including the G_q, G_i and G_s proteins. The α-ARs and β-ARs can couple to more than one G-protein, however α_1-ARs and α_2-ARs usually couple with G_q (stimulation of phopholipase C) and G_i (inhibition of adenylyl cyclase) respectively while the β-ARs usually couple with G_s (stimulation of adenylyl cyclase) (Small *et al*. 2003). The G_s-protein is a heterotrimer consisting of an α-, β- and γ-subunit (see section 2.2.8, p111 for more information) (McGraw & Liggett 2005, Oldham & Hamm 2006).

The role of the β-ARs in WAT lipolysis can be described as follows (Figure 2.5):

In the basal state (Figure 2.5a), when nutrients are abundant (e.g. after consumption of food), adipocytes synthesize and also take up non-esterified fatty acids (NEFAs). In both WAT and brown adipose tissue (BAT) these NEFAs are esterified and stored inside the lipid droplets in the form of triacylglycerol (TAG). The amount that is stored reflects the range of positive energy balance over time determined by energy intake minus energy expenditure (Peters 2006). In the WAT, the cyclic adenosine monophosphate (cAMP) dependent protein kinase (PKA) is bound to an AKAP anchor protein on the WAT cell membrane. In the cytosol the nonphosphorylated hormone sensitive lipase (HSL) is bound to cytosolic acceptors such as lipotransin. For HSL to have access to the lipid droplet to hydrolyse the stored TGs, it must first be phosphorylated and then be translocated to the surface of the droplet. Furthermore, nonphosphorylated perilipin is tightly bound to the surface of the lipid droplet, protecting the stored TG against lipolysis (Robidoux *et al*. 2004).

Lipolysis (Figure 2.5b) is triggered by a drop in blood glucose levels which occurs either a few hours after ingestion of food or during an overnight fast or when a negative energy balance exists (in response to decreased food intake, fasting, or sustained increased physical activity). This decrease in blood glucose levels triggers the sympathetic nervous system to release the catecholamines epinephrine and norepinephrine (Robidoux *et al*. 2004). The release of catecholamines is also stimulated by exercise, with the stimulation increasing markedly as the intensity of the exercise increases to 50% maximal exertion and above (Snyder *et al*. 2008). The released catecholamines interact with the β-ARs on the cell membranes of WAT, which then couple to G_s and G_i proteins. G_s coupling leads to stimulation of adenylyl cyclase and PKA, which is consequently released from the AKAP anchor protein into the cytosol. PKA then phosphorylates two serine residues (Ser-659 and Ser-660) of HSL and six serine residues (Ser-81, Ser-222, Ser-276, Ser-433, Ser-494, and Ser-517) of perilipin A. The phosphorylated HSL can now translocate to the surface of the lipid droplet. On the surface of the lipid droplet the tight association of Perilipin A is lost when it becomes phosphorylated. Consequently, HSL can access the inside of the droplet and stimulate lipolysis (Snyder *et al*. 2008, Robidoux *et al*. 2004). Furthermore, elevated glucocorticoids stimulate the transcription of a TAG lipase named denustrin/ATGL. The stored TAG in the lipid droplet will firstly be hydrolyzed to diacylglycerol (DAG) and a fatty acid by denustrin/ATGL. DAG is then hydrolyzed by HSL to monoacylglycerol (MAG) and a second fatty acid. MAG is lastly hydrolyzed by MAG-lipase to glycerol and a third fatty acid (Ahmadian *et al*. 2010). G_i activation also leads to EGF receptor transactivation and activation of the ERK pathway including the ERK1/2 MAP kinase and p38 MAP kinase. Activation of p38 MAPK increases cAMP levels and PKA activity. Therefore the ERK pathway can also lead to the phosphorylation of a different serine residue on HSL (Ser-600) and probably phosphorylation of perilipin as well. However, lipolysis through the ERK pathway appears to account for only 15-25% of total lipolysis, with the G_s coupled pathway accounting for most (Robidoux *et al*. 2004).

It is clear that catecholamines are important regulators of lipolysis in WAT and act by stimulating the β-ARs to induce lipolysis (Robidoux *et al*. 2004). The β-AR genes are therefore important candidate genes to consider for obesity development as a variation in these genes might affect the functionality of the receptor that may alter

adipocytes lipolysis. It has been shown that mice without β-ARs displayed reduced metabolic rate and were slightly obese on a normal chow diet. However, on a high fat diet, these mice developed massive obesity compared to wild-type mice (Bachman *et al*. 2002). Furthermore, a decreased *ADRB3* mRNA expression that has been reported in all models of congenital obese mice and rats including the leptin-deficient *ob/ob* mice, *db/db*, *tubby*, *fat* and the Zucker fatty rat is responsible for the inability to mobilize stored fat in response to a β-agonist (reviewed by Robidoux *et al*. 2004).

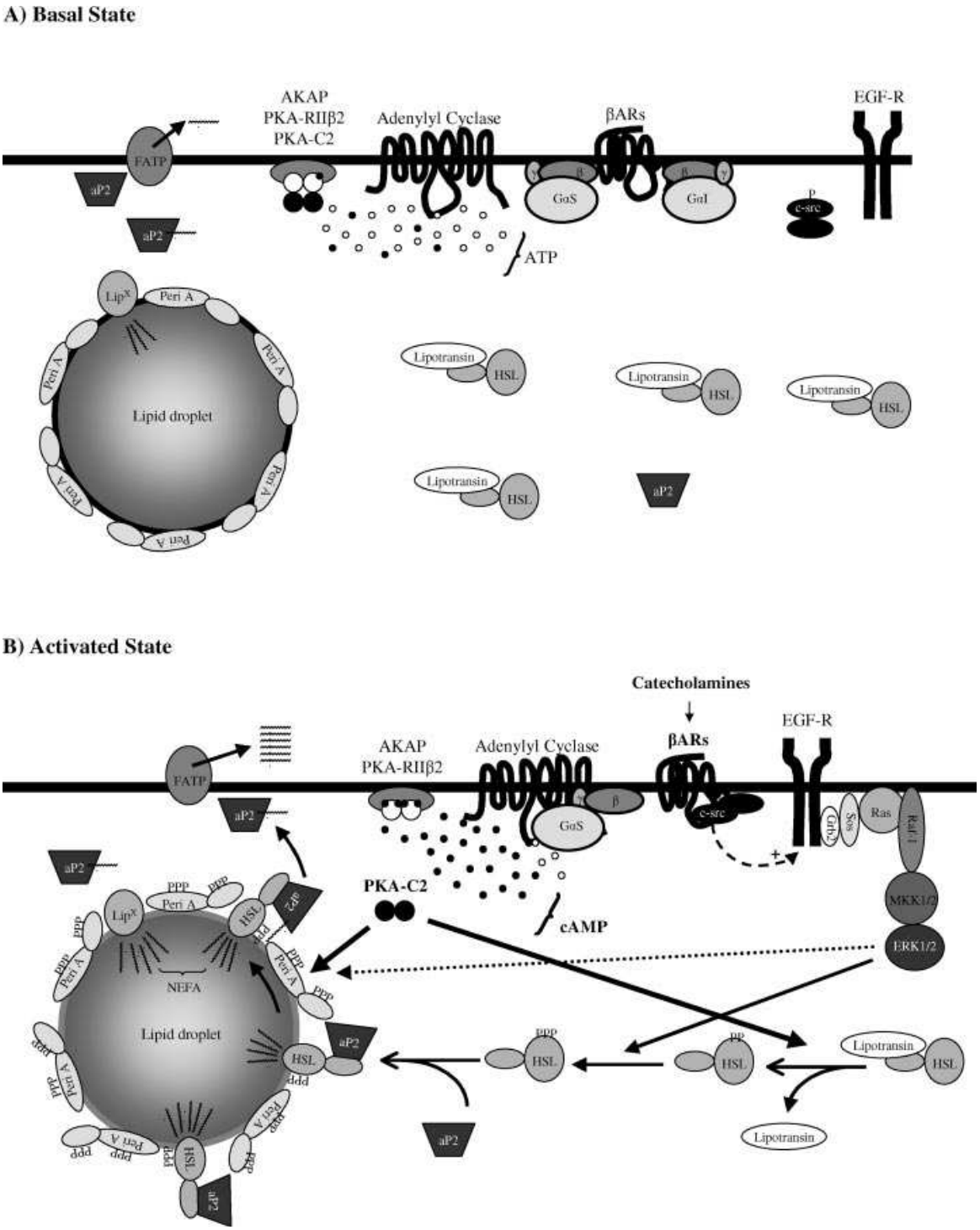

Figure 2.5: Mechanism of WAT lipolysis stimulation by ADRB3 in two stages namely the basal state (Figure 2.5a) and lipolysis stage (Figure 2.5b) (from Robidoux *et al*. 2004).

2.6.4 The *ADRB3* Trp64Arg polymorphism

In 1995, three groups independently reported a missense mutation in the first transmembrane domain or the most proximal residue of the first intracellular loop (Figure 2.4) of the human β_3-adrenergic receptor. The mutation produces a Thymine (T) to Cytosine (C) transition at nucleotide position 190, causing the replacement of tryptophan (Trp) by arginine (Arg) at amino acid position 64 (Trp64Arg) (Clément *et al*. 1995, Walston *et al*. 1995, Widén *et al*. 1995).

Although the exact functional effect of the Trp64Arg polymorphism on the expression and activity of the *ADBR3* gene is still unclear, *in vitro* research indicates that the Trp64Arg polymorphism might cause an expression of a β_3-adrenergic variant receptor (Kurokawa *et al*. 2008, Small *et al*. 2003). This has been demonstrated in murine pre-adipocytes (Kimura *et al*. 2000), Chinese hamster ovary cells and human embryonic kidney cells (Pie´tri-Rouxel *et al*. 1997). Stimulation of rodent and human cell lines with the Arg64-allele results in a reduced amount of cAMP released after catecholamine stimulation, a reduced ability to stimulate adenyl cyclase activity (Kimura *et al*. 2000, Pie´tri-Rouxel *et al*. 1997) and a reduction in lipolysis (Umekawa *et al*. 1999). It appears that the fact that the Arg64 and Trp64 variants of ADBR3 prefer different G-proteins to couple with (Small *et al*. 2003), could explain these findings. When compared to the wild-type Thr64 ADRB3, the mutant Arg64 receptor couples less efficiently with the Gs protein after catecholamine stimulation which results in the release of less cAMP (Pie´tri-Rouxel *et al*. 1997). PKA is dependent on cAMP for activation and therefore decreased phosphorilation of HSL and perilipin occurs with decrease cAMP levels, resulting in the decrease in lipolysis seen in Arg64 adipocytes (Pie´tri-Rouxel *et al*. 1997, Umekawa *et al*. 1999). However, although these *in vitro* findings have been published by some (Pie´tri-Rouxel *et al*. 1997, Umekawa *et al*. 1999, Kimura *et al*. 2000), others have failed to observe any *in vitro* functional effects (Candelore *et al*. 1996, Li *et al*. 1996). Furthermore, it has been shown that the Arg64 variant has a decreased sensitivity to ADRB3 agonists and this may also result in lower signal transduction accompanied by decreased lipolysis (Hoffstedt *et al*. 1999).

2.6.5 Genotype and allele frequencies

The frequency of the wild-type Trp64Trp genotype is higher in Caucasian populations ranging between 82 and 90%, lower in Asian populations ranging between 65 and 70%, intermediate in African-Americans (75%) and Jamaicans (81%), while the lowest frequencies were observed in South African Blacks and Pima Indians at 48% and 46% respectively (Table 2.24). The Arg64Arg genotype is virtually absent in Caucasian populations while between one and 7% of African or Asian populations have this genotype, with the highest frequency (9%) reported for Pima Indians. The frequency of the mutant Arg64-allele is therefore lower in Caucasians, intermediate in Asians and high in South African Blacks and Pima Indians.

Table 2.24: Genotype frequencies of the *ADRB3* Trp64Arg polymorphism and frequency of the mutant Arg64-allele.

Population	Weight and diseases status	n	Trp/ Trp %	Arg/ Trp %	Arg/ Arg %	Arg64-allele %	References
Caucasian							
Finnish	Obese	170	81	19	0		Valve *et al*. 1998
Finnish	DM2	119	87	13	0	8	Pulkkinen *et al*. 1999
	Non-DM2	185	84	16	0	7	
Spanish		1063	90	10	0	5	Corella *et al*. 2001
Spanish	With OSAS	387	82	17	1	10	Pierola *et al*. 2007
	Without OSAS	137	85	15	0	8	
Spanish ♀		172	90	10	0	5	Ramis *et al*. 2004
♂		160	87	13	0	7	
Austrian ♀		179	83	17	0	9	Festa *et al*. 1999
Dutch ♀		1519	88	12	0	6	Zafarmand *et al*. 2008
German		988	82	17	1	9	Stangl *et al*. 2001
European		4854	86	13	1		Kurokawa *et al*. 2008
French Canadian		743	85	15	0	8	Ukkola *et al*. 2000
USA		156	84	15	1		Lima *et al*. 2007
African							
South African Black	Insulin resistant	102	48	47	5	28	Van Rooyen *et al*. 2008
Jamaicans		586	81	18	1	11	McFarlane-Anderson *et al*. 1998
African-American		72	75	21	4		Lima *et al*. 2007
Native American							
Pima Indian		642	46	45	9	31	Walston *et al*. 1995
Asian							
Chinese		695	69	28	3	17	Hao *et al*. 2004
Japanese		287	65	32	3	19	Tsuzaki *et al*. 2007
Japanese		261	68	28	4	18	Shima *et al*. 1998
Japanese		191	67	28	5	19	Kadowaki *et al*. 1995
Japanese		106	70	24	6		Kahara *et al*. 2002
Japanese		120	71	23	6	20	Murata *et al*. 2003

USA = United States of America, DM2 = Type 2 diabetes, OSAS = obstructive sleep apnoea syndrome

2.6.6 Associations with obesity related phenotypes.

Weight, BMI, obesity and measures of body fat distribution and content

According to the latest Human Obesity Gene Map (Rankinen *et al*. 2006), numerous human studies have reported positive associations between the *ADRB3* mutant Arg64-allele and obesity, early onset obesity, higher weight, BMI, abdominal subcutaneous fat, abdominal visceral fat, hip circumference, fat mass, waist circumference, body fat percentage and waist-to-hip ratio (Rankinen *et al*. 2006, Pierola *et al*. 2007). Three independent studies also indicated an association between the Arg64-allele and a higher body weight increase over 5 years (Matsuoka *et al*. 2004), 20 (Clement *et al.* 1995) and 25 years (Nagase *et al*. 1997). Many other studies have however failed to find any associations with the above-mentioned obesity related phenotypes and the Arg64-allele (Rankinen *et al*. 2006).

Four meta-analyses have been published regarding the association between the *ADRB3* Trp64Arg polymorphism and BMI (Table 2.25). The first meta-analysis showed no association between this polymorphism and BMI in 7399 individuals from different ethnicities reported in 23 studies published before June 1997 (Allison *et al*. 1998).

Subsequent meta-analyses (Fujisawa *et al*. 1998, Kurokawa *et al*. 2001, Kurokawa *et al*. 2008) indicated that Arg64-allele carriers have a significantly higher BMI in comparison with wild-type Trp64Trp homozygotes. The most recent meta-analysis including 97 studies and 44833 individuals indicated that the Arg64-carriers had a 0.24kg/m^2 higher BMI compared to the Trp64Trp homozygotes (Kurokawa *et al*. 2008). Although the meta-analysis by Fujisawa *et al*. (1998) showed that the association between the Arg64-allele and a higher BMI was applicable to all ethnicities included, the last two meta-analyses point towards associations being present only in Asian populations.

Table 2.25: Meta-analyses of the association between the *ADRB3* Trp64Arg polymorphism and BMI.

Nr of studies	Date studies were published	Nr of subjects	Ethnicity	Results	Author
23	Before June 1997	7399	different ethnicities	No significant association. No effect of heterogeneity by ethnicity.	Allison *et al*. 1998
31	Before January 1998	9236	different ethnicities	Arg64 allele carriers had a higher BMI (0.30kg/m^2) compared with Trp64 homozygotes. No effect of heterogeneity by ethnicity.	Fujisawa *et al*. 1998
27	Before January 2001	6582	Japanese only	Arg64 allele carriers had a higher BMI (0.26kg/m^2) compared with Trp64 homozygotes.	Kurokawa *et al*. 2001
97	Before May 2007	44833	Caucasian =European East Asian =Japanese	Arg64 allele carriers had a higher BMI (0.24kg/m^2) compared with Trp64 homozygotes. Heterogeneity effects: significant difference (0.31kg/m^2) in the East Asian subgroup but not in the European descendents subgroup. No difference between Asian and European subgroups.	Kurokawa *et al*. 2008

2.6.7 Associations with health indicators

Indicators of glucose and insulin homeostasis, blood lipid profile and blood pressure

A meta-analysis on the associations between the *ADRB3* Trp64Arg polymorphism and obesity related co-morbidities that was published in 2005 by Zhan and Ho, found that the Arg64-allele was associated with higher fasting insulin levels in Asians but not Caucasians (Zhan & Ho 2005). In the most recent meta-analysis published on this topic (Zafarmand *et al*. 2008) no association with acute myocardial infarction or coronary heart disease risk and the *ADRB3* Trp64Arg polymorphism was found. The Arg64-allele has also been associated with early onset type-2 diabetes (Walston *et al*. 1995), diabetic retinopathy and nephropathy (Sakane *et al*. 1997a, Sakane

et al. 1998). Although inconclusive, some studies also reported on associations with hypertension, while others do not support such associations (summarized by Rozec & Gauthier 2006).

2.6.8 Associations with weight loss outcomes

The association between the *ADRB3* Trp64Arg polymorphism and weight loss outcome has been investigated in 13 weight loss interventions of which four were performed in Caucasians, seven in Japanese, one in Koreans and one in Chinese children (Table 2.26). In the Caucasian populations, two 12-month interventions and one three-month weight loss intervention showed that the Trp64Trp homozygotes and Arg64-allele carriers experienced similar weight reductions (Tchernof *et al*. 2000, Rawson *et al*. 2002, De Luis *et al*. 2007). The authors did however argue that the wild-type Trp64Trp homozygotes benefited more from these interventions as they experienced greater decreases in visceral adipose tissue, total cholesterol-to-HDL ratio (Tchernof *et al*. 2000) and greater decreases in glucose, insulin resistance (De Luis *et al*. 2007). The fourth weight loss intervention involving a Caucasian population consisted of a 12-month programme. With this programme, Finnish obese subjects attended weekly group sessions with a nutritionist and followed a weight loss diet for 12 weeks followed by a weight maintenance period of 40 weeks. These authors indicated that subjects who were carriers of the mutant *ADRB3* Arg64 allele and the mutant *UCP1* A/G allele were not able to maintain their weight loss during the 40-week weight maintenance phase (Fogelholm *et al*. 1998). Therefore, such subjects may benefit from more intensive interventions to induce long-term weight loss maintenance.

Four of the seven weight loss interventions carried out in Japanese showed that wild-type Trp64Trp homozygotes experienced greater weight losses after following low energy diets and exercise recommendations (Kogure *et al*. 1998, Yoshida *et al*. 1995, Sakane *et al*. 1997b) or the medication Mazindol (Shimizu *et al*. 2007) for three months. Although the other three interventions indicated that the *ADRB3* Trp64Arg polymorphism did not influence weight loss outcome, it was also proposed that wild-type Trp64Trp homozygotes might benefit more from a conservative weight loss programme to decrease not only their weight but also other variables such as visceral fat area and visceral-to-subcutaneous fat ratio (Nakamura *et al*. 2000). A similar argument was applicable to the Koreans after following a three months weight loss intervention. Although the BMI of both genotype groups decreased similarly, the Trp64Trp homozygotes experienced better decreases in total, visceral and subcutaneous fat areas and improvements in blood lipid levels and glucose metabolism (Kim *et al*. 2003). Finally, Trp64Trp homozygous Chinese obese children benefited more from a three-month intervention aiming at decreasing their fat and cholesterol intake to limit weight gain (Xinli *et al*. 2001).

It is thus evident that subjects with Caucasian or Asian ancestry who are *ADRB3* Trp64Trp homozygotes may either have more successful weight loss or weight maintenance following a weight loss intervention or may experience additional improvements in associated health variables. However, the mutant Arg64-allele carriers may find it more difficult to lose weight or successfully maintain weight loss and may need more intensive interventions to secure sustained health effects.

Table 2.26: The association between the *ADRB3* Trp64Arg polymorphism and change in weight (BMI) and other variables following a weight loss intervention.

Population	Sample size	Genotype frequencies	Inclusion criteria	Weight loss intervention	Variables measured	W64W = ↑ weight loss	Results	Reference
Finland	n=85 ♀	W64W=84% R64=16%	BMI>29	***12 months intervention:*** ▪ Weight loss phase (12 weeks) - Week 1 & 10-12 = 4200 - 4600 kJ/ day - Weeks 2-9 = VLCD (40% of REE) 2700 kJ/ day ▪ Maintenance phase (40 weeks) ▪ Weekly small group sessions with nutritionist all 12 months	***Measured at 0, 3 & 12 months:*** ▪ BMI, FM, ▪ REE, adjusted REE, VO_2 max, insulin.	**Yes**	Combined effect of *ADRB3* W64R and UCP1 A/G Compared 4 groups: 1) subjects with no mutations for both genes, 2) with R64; 3) UCP1 mutation, 3) both mutations. ***Between group differences:*** ▪ No baseline differences ▪ No difference after 3 months weight loss phase ▪ During 9 months weight maintenance phase: - ↑ BMI in both mutations group vs. ↓ BMI in no mutations group ($p<0.05$) - Over whole 12 months: Both mutations group loss less weight than no mutations group	Fogelholm *et al*. 1998
USA ♀ Caucasian	n=24	R64R=4%; W64R=42% W64W=54%	BMI≥27 post-meno-pausal ♀	***13.5 months intervention:*** ▪ First 1.5 months = weight stable period ▪ Next 12 months: 5000 kJ / day AHA step 2 diet. ▪ Dietitian visits. ▪ Encouraged not to change physical activity habits. ▪ With or without modified fasting supplements	***Measured at 0 and 13.5 months:*** ▪ DEXA: FM, FFM ▪ CT scanner: VAT SAT, ▪ TC, TG, HDL, LDL, ▪ Hyperinsulinemic-euglycemic clamp: glucose disposal, clamp insulin levels, EGP	**No**	***After intervention both genotype groups (W64W and R64 carriers) experienced:*** ▪ ↓ BMI, FM, FFM, VAT, SAT, TC:HDL ratio, clamp insulin, basal glucose, clamp glucose and improved glucose disposal rates. ***R64 carriers:*** ▪ Lower ↓ in visceral adipose tissue ▪ Lower ↓ TC:HDL ratio	Tchernof *et al*. 2000
USA ♀: ethnicity unknown	n=34 Drop-out = 35	W64W=56% R64R+ R64W=44%	BMI≥27 post-meno-pausal ♀	***13.5 months intervention:*** ▪ First 1.5 months = weight stable period ▪ Next 12 months: 5000 kJ / day AHA step 2 diet. ▪ Dietitian visits. ▪ With or without modified fasting supplements	***Measured at 0 and 13.5 months:*** ▪ BMI. ▪ Indirect calorimetry: TEF, RMR, RQ ▪ Doubly labelled water dose: after 10 days = determine TEE, % body fat, FFM, FM, PAEE.	**No**	▪ No difference in SNP between drop-outs and subjects completed the study. ▪ After intervention total sample experienced: ↓ BMI, % body fat, FFM, FM, RMR ▪ After intervention no significant differences between W64W and R64 carriers.	Rawson *et al*. 2002

Table 2.26: *continued*

Population	Sample size	Genotype frequenies	Inclusion criteria	Weight loss intervention	Variables measured	W64W = ↑ weight loss	Results	Reference
Spain	n=65 ♀= 47 ♂= 18	W64W=85% W64R=15%	BMI≥30	***3 month intervention:*** ▪ Diet = 6384 kJ/ 1520 kCal, 52% carbohydrates, 25% fat, 23% proteins. ▪ Exercise: 60 min aerobic 3×/week	***Measured at 0, 3 months:*** ▪ BMI, WC, WHR, FM, FFM. ▪ Energy, carbohydrate, protein, fat intake. ▪ BP, TC, HDL, LDL, TG, BG, insulin, HOMA-IR, lipoprotein (a), CRP, Adipocytokines: leptin, adiponectin, resistin. ▪ RMR, VO_2 max.	**No**	% of responders (those who lost weight) was similar in W64W (89%) and W64R (80%) groups ***Difference between groups at 0 & 3 months:*** ▪ W64R = ↑ CRP ***Change over 3 months within genotype groups:*** ▪ W64W = ↓ BMI, FM, WC, SBP, glucose, HOMA-IR, leptin and ↑ RMR, RMR/FM ratio, exercise, VO_2 ▪ W64R = ↓ BMI, FM, WC, SBP, leptin and ↑ RMR, RMR/FM ratio, VO_2	De Luis *et al*. 2007
Japanese	n=24 ♀= 17 ♂= 7	W64W=67% W64R=33%	BMI≥35	***3 months intervention:*** Previously unsuccessful pt in 3 month low kJ diet & 60min PA/d received treatment with Mazindol for 3 months	***Measured at 0 & 3 months*** ▪ Weight, % fat, BP	**Yes**	W64W = greater ↓ in weight & BP.	Shimizu *et al*. 2007
Japanese	n=88 ♀	W64W=60% R64W=34% R64R=6%	BMI≥30	***3 months intervention:*** Low energy diet and exercise regime	***Measured at 0, 3 months:*** ▪ weight, height, BMI	**Yes**	W64W = greater weight loss	Yoshida *et al*. 1995
Japanese ♀	n=61	W64W=61% W64R=34% R64R=5%	BMI≥30 ♀ with DM2	***3 months intervention*** ▪ Low calorie diet and exercise	▪ BMI, WHR. ▪ Adjusted resting metabolic rate. ▪ Fasting blood glucose, insulin, insulin resistance index, HbA1C, ▪ % body fat (skinfold measurements)	**Yes**	***Baseline differences between groups*** ▪ R64 carriers = ↑ body fat %, WHR, glucose, insulin, HOMA-IR, HbA1c ***Change within groups over 12 weeks:*** ▪ W64W = ↓ BMI, WHR, glucose, HOMA-IR, HbA1c ▪ R64 carriers = ↓ BMI, WHR, glucose ***Difference between groups for change in variables:*** ▪ R64 carrriers = smaller ↓ in BMI, WHR, glucose, HbA1c compared to W64W	Sakane *et al*. 1997b

Table 2.26: *continued*

Population	Sample size	Genotype frequenies	Inclusion criteria	Weight loss intervention	Variables measured	W64W = ↑ weight loss	Results	Reference
Japanese	n=90 ♀	W64W=56% R64W=39% R64R=5%	BMI≥26.4	***3 months intervention:*** ▪ Diet = 5880 kJ/d, 1.5 g/kg protein, 30g fat, 20g fibre. ▪ Exercise = walking 60min 3-7× /week ▪ Individualized dietary advice by dietitian.	***Measured at 0 & 3 months*** ▪ BMI, weight, % fat, FM, MRI:TFA, VFA, SFA, V/S ▪ Glucose, insulin, TC, HDL, TG	**No**	***Baseline differences between W64W & R64 carriers*** Post-menopausal women: ▪ W64W = ↓ BMI, weigh, FM, TFA, SFA. ***Difference between W64W and R64 carriers for change in variables over 3 months:*** ▪ Premenopausal ♀: W64W = greater ↓ in V/S ratio and HDL ▪ No difference in post-menopausal ♀ ▪ All ♀: W64W = greater ↓ in VFA and V/S ratio	Nakamura *et al*. 2000
Japanese ♀	n=76	W64W=64% W64R=32% R64R=4%	BMI>21	***3 month intervention*** Behavioural weight-loss programme: ▪ Diet ▪ Exercise: counting step/day with pedometer ▪ Supportive group therapy ▪ Health check to recognize risks ▪ Intensive individual sessions with professional to address unhealthy lifestyle, weekly weigh-ins	▪ Body fat, WC, HC, triceps skin fold. ▪ Indirect calorimetry: REE, ▪ Blood pressure, TC, TG, phospoholipid, NEFA, glucose, insulin, leptin, HOMA-IR ▪ FFQ, PA questionnaire, ▪ bio-electrical impedance,	**No**	***Change over 3 months within genotype groups:*** ▪ W64W: ↓ kJ, prot, fat /day, BMI, WC, HC, BP, LDL:HDL ratio, phospholipid and ↑ in body fat %, HDL, nr of steps/day. ▪ Correlation between weight change & change in kJ intake & change in nr of steps (PA) ▪ R64 carriers: ↓ kJ, fat / day, HC, LDL, LDL:HDL ratio, phospholipid and ↑ in body fat %, HDL (No correlations) **No significant differences for change in these variables between W64W and R64 carriers.**	Shiwaku *et al*. 2003
Japanese	n=37	W64W=67% R64W=30% R64R=67%	BMI≥23	***6 months intervention:*** ▪ Trained nutritionists & nurses taught 7-10 diet, exercise and behaviour modification lessons on individualized basis to each pt ▪ Health check to recognize risks.	***Measured at 0 & 6 months*** ▪ Weight, BMI, VFA, SFA, ▪ Glucose, 2h glucose, HbA1c, insulin, HOMA-IR. ▪ TC, LDL, TG.	**No**	***Change over 6 months within genotype groups:*** ▪ W64W = ↓ weight, BMI, SFA, HbA1c. ▪ R64 carriers = ↓ weight, BMI, glucose, HbA1c. ▪ No difference between genotype groups for change in variables over 6 months	Kuriyama *et al*. 2008

Table 2.26: *continued*

Population	Sample size	Genotype frequenies	Inclusion criteria	Weight loss intervention	Variables measured	W64W = ↑ weight loss	Results	Reference
Japanese	n=113 ♀	W64W=66% R64W=30% R64R=4%	BMI≥30	***3 months intervention:*** ▪ Low kilojoules diet and exercise regime	***Measured at 0, 3 months:*** weight, height, BMI	**Yes**	Combined effect of *ADRB3* T64A and UCP1 A/G: A64 carriers + mutant UCP1 = lost less weight	Kogure *et al*. 1998
Korean	n=70 ♂	W64W=71% R64W=29% R64R=0%	BMI≥25 / WHR>0.9/ % body fat>24% and CAD	***3 months intervention:*** ▪ Goal = 5% weight loss ▪ Diet = 300 kCal/d less than usual ▪ 60% carbohydrate, 20% protein, 20% fat. ▪ Weekly individual sessions with dietitian ▪ 3-day dietary records & daily activities records for 3 months.	***Measured at 0, 3 months:*** ▪ BMI, WHR, BP, Newly onset DM %, HT%, smoking. ▪ TE, protein, fat carbohydrate, ▪ Basal & 2h glucose, FFA, Insulin, C-peptide. ▪ TG, TC, HDL, LDL, athrogenic index, TC/HDL, LDL/HDL, ApoAI, ApoB. ▪ At L1 & L4: TFA, VFA, SFA V/S ratio, Calf fat & muscle area, Mid-thigh fat & muscle area	**No**	▪ Similar weight loss in both genotype groups ***Change over 3 months within genotype groups:*** T64T = ↓ BMI, SBP, energy intake, ↓ TFA, VFA, SFA at L1, L4 ↓ insulin, 2h glucose, FFA, C-peptid ↓TG, TC, LDL, athrogenic index, TC/HDL, LDL/HDL, ApoB T64A = ↓ BMI, SBP, energy intake, mid thigh fat	Kim *et al*. 2003
Chinese children	n=47 ♀= 16 ♂= 31 n=36: obese intervention group n=11: obese controls	In school sample n=311: W64W=67% W64R=31% R64R=2%	obese children	***3 months intervention:*** ▪ Parents & children receive lecture on nutrition & health. ▪ Parents received: nutrient information, examples of diets and menus for children. ▪ Diet aims to decrease fat and cholesterol intake ▪ Diet = based on USA NCEP Step I diet ▪ Individualized instruction from nutritionist with: - Family visits 1/months - Telephone calls 1/week ▪ Control group received none of the above.	***Measured at 0, 3 months:*** ▪ weight, height, BMI, sum of 3 skinfolds (subscapular, biceps, triceps), BP. ▪ From 3 day food diaries: TE, fat%, cholesterol	**Yes**	▪ Intervention group had a ↓ TE, fat %, cholesterol intake ▪ Difference between W64W and R64 carriers in intervention and all controls for change in variables over 3 months: ▪ W64W = lower increase in weight & BMI vs. R64 carriers and controls who had similar changes in these variables.	Xinli *et al*. 2001

R54R = Arg64 homozygotes, R64W = heterozygotes, W64W = Trp64 homozygotes, W64 = Trp64 allele carriers, BMI = body mass index, WC = waist circumference, HC = hip circumference, WHR = waist-hip ratio, BW = body water, FM = fat mass, FFM = fat free mass, BP = blood pressure, SBP = systolic blood pressure, TC = total cholesterol, HDL = high density lipoprotein, LDL = low density lipoprotein, TG = triglycerides, RMR = resting metabolic rate, BG = blood glucose, VWAT = visceral white adipose tissue, SWAT = subcutaneous white adipose tissue, TFA = total fat area, VFA = visceral fat area

2.7 β2 Adrenergic receptor (β_2-AR and *ADRB2*; β_2-adrenoceptor) gene

2.7.1 The *ADRB2* gene

The human β_2-adrenergic receptor gene (*ADRB2*) was first cloned in 1987, assigned to the long arm of chromosome five and localized to position 5q31-32 using somatic cell hybrids and in situ hybridization respectively (Kobilka *et al*. 1987a). The intronless *ADRB2* gene consists of 1242 bp and has only one exon (Kobilka *et al*. 1987a, Kobilka *et al*. 1987b). *ADRB2* also contains a short open reading frame located 102 bp upstream of the receptor coding block referred to as the 5' leader cistron (Parola & Kobilka 1994).

2.7.2 The ADRB2 protein

The *ADRB2* gene encodes a 413 amino acid β_2-Adrenergic receptor and a 19 amino acid leader cistron peptide, which regulates the amount of *ADRB2* expressed in a cell by obstructing its mRNA translation (Parola & Kobilka 1994, Liggett 2000). The structure of the β_2-Adrenergic receptor is similar to that of the other GPCRs as depicted in Figure 2.4, p87.

The ADRB2 protein is abundant in human WAT and BAT (Robidoux *et al*. 2004). It is also expressed in the human heart including the atria, ventricles and sinoatrial node as well as throughout the lungs from the trachea to the alveoli including the airway smooth muscle, lung epithelial and endothelial cells, type II cells and mast cells (Johnson 2006, Snyder *et al*. 2008).

2.7.3 Physiological function of ADRB2

The β_2-adrenoceptors are primarily stimulated by epinephrine to increase lipolysis (as described in section 2.6.3, p87), bronchodilation, vasodilation and ventricular function (Snyder *et al*. 2008). The specific involvement thereof in the lungs and heart to increase bronchodilation, vasodilation and ventricular function is not discussed as it is beyond the scope of this review, but is reviewed in several articles (McGraw & Liggett 2005, Johnson 2006, Snyder *et al*. 2008).

2.7.4 The *ADRB2* Arg16Gly polymorphism

The mutation in *ADRB2* produces an Adenine (A) to Guanine (G) transition at nucleotide position 46, causing the replacement of arginine (Arg) by glycine (Gly) at amino acid position 16 (Arg16Gly) (Green *et al*. 1994, Green *et al*. 1995, Large *et al*. 1997). This polymorphism is found in the extracellular amino terminus of the ADRB2 protein (see Figure 2.4, p87).

In vitro studies have indicated that the Arg16Gly polymorphism can alter the ADRB2 function and consequently influence lipolysis regulation in subcutaneous fat cells (Green *et al*. 1994, Green *et al*. 1995, Large *et al*. 1997, Hoffstedt *et al*. 2001). *In vitro* experiments on abdominal subcutaneous adipocytes obtained from 140 Caucasian women illustrated a five-fold increase in agonist sensitivity in Gly16 carriers without any change in *ADRB2* expression or maximum lipolytic action (Large *et al*. 1997). In contrast, a 10-fold decrease in ADRB2 agonist sensitivity was observed in subcutaneous adipocytes of subjects carrying the mutant alleles, Gly16-Glu27- and Ile164, of three *ADRB2* polymorphisms (Hoffstedt *et al*. 2001).

The Arg16Gly polymorphism has been associated with altered agonist-promoted downregulation of *ADRB2* expression, causing altered cellular trafficking and desensitization of the receptor (Green *et al*. 1994, Green *et al*. 1995, Moore *et al*. 2000). The *ADRB2* Gly16-allele has been associated with a higher decrease in *ADRB2* expression in adipose tissue due to enhanced down-regulation after prolonged agonist stimulation (Green *et al*. 1994, Green *et al*. 1995). This may consequently result in reduced receptor function and lower efficiency of lipolysis stimulation, leading to excess fat accumulation over time (Ellsworth *et al*. 2002). Consequently, individuals carrying the Gly16-allele (especially homozygotes) may be more likely to become obese (McGraw *et al*. 1998, Ellsworth *et al*. 2002). It is also apparent that the Gly16-allele may inhibit lipolysis to a greater degree in abdominal adipose tissue than in gluteal-femoral tissue. Ellsworth *et al*. (2002) proposed that males carrying the Gly16-allele might be unable to compensate effectively to increase lipolysis due to higher abdominal α2-AR efficiency, which also inhibits lipolysis.

Jocken *et al*. (2007) measured the effect of stepwise infusion of increasing doses of the non-selective β-agonist isoprenaline (ISO) on *in vivo* lipolysis and fat oxidation in 65 male and 43 female overweight and obese subjects. During ADRB2 stimulation with ISO, Gly16Gly women experienced a higher increase in circulating free fatty acids and glycerol compared to Arg16-allele carriers. This enhanced lipolytic response was accompanied by increased fat oxidation after ISO stimulation in Gly16Gly homozygous women compared to a decrease in fat oxidation in Arg16-allele carriers (Jocken *et al*. 2007). These effects were not as pronounced in male overweight subjects. In the latter subjects the Gly16Gly genotype was only associated with increased circulating free fatty acids and not with glycerol or fat oxidation levels (Jocken *et al*. 2007). Similarly, Gly16 female allele carriers had higher fasting free fatty acid levels and higher increases in free fatty acids following an oral glucose tolerance test (Meirhaeghe *et al*. 2001).

2.7.5 Genotype and allele frequencies

The frequency of the *ADRB2* wild-type Arg16Arg genotype ranges between 10 and 29% in Caucasians, while that of the mutant Gly16Gly genotype ranges between 32 and 53% (Table 2.27). Lower ranges for the Gly16Gly genotype are found in subjects with African (26 to 31%) or Asian ancestry (9 to 33%). This Gly16Gly genotype frequency range does not include frequencies for subjects with Asian ancestry of 59%, 49% and 57% reported

respectively for overweight subjects who had blood pressure or weight increases over a five year follow-up period (Kawaguchi *et al*. 2006) or normal weight subjects with high norepinephrine levels (Masuo *et al*. 2007).

Although the Gly16-allele is the mutant variant of the *ADRB2* gene, it is actually not found as the minor allele frequency in most populations. The Gly16-allele was found at a frequency of 0.55 to 0.66 in Caucasians, 0.36 to 0.55 in subjects with African ancestry and 0.35 to 0.57 in subjects with Asian ancestry.

Table 2.27: Genotype frequencies of the *ADRB2* Arg16Gly polymorphism and frequency of the mutant Gly16-allele.

Population	Weight and disease status	n	Arg/ Arg %	Arg/ Gly %	Gly/ Gly %	Gly16 allele	References
Caucasian							
Scandinavian ♀	BMI>25	141	19	45	36		Eriksson *et al*. 2004
Danish		2904	14	47	39	0.62	Gjesing *et al*. 2007
Sweden ♂	non-obese	180	19	44	37	0.59	Ehrenborg *et al*. 2000
Sweden		267	29	31	39	0.55	Rosmond *et al*. 2000
Sweden	Obese	82	17	38	45	0.64	Large *et al*. 1997
	Non-obese	58	22	24	53	0.66	
Austrian ♀	Obese	183	20	45	35	0.57	Oberkofler *et al*. 2000
	Non-obese	216	17	47	36	0.60	
Caucasian							
French-Canadian		334	17	39	44	64	Ukkola *et al*. 2000
France		836	14	48	38	63	Meirhaeghe *et al*. 2000
France ♂		567	13	50	37		Dallongeville *et al*. 2003
France ♀		562	15	46	39		Dallongeville *et al*. 2003
Netherlands: ♂	weight gain over 7 yrs	134	16	40	44		Van Rossum *et al*. 2002
	weight stable over 7 yrs	138	10	55	36		
♀	weight gain over 7 yrs	152	12	53	35		
	weight stable over 7 yrs	158	10	44	46		
Italians	Random population	305	16	41	43		Pinelli *et al*. 2006
	DM2	342	20	44	36		
German children	Obese	296	18	46	40	59	Tafel *et al*. 2004
	Lean	134	17	44	39	61	
UK		604	12	44	44	65	Meirhaeghe *et al*. 2001
American		816	12	47	41	64	Ellsworth *et al*. 2002
American		161	21	37	42		Lima *et al*. 2007
American ♀		538				58	Terra *et al*. 2005
European Brazilians		334	19	49	32	56	Mattevi *et al*. 2006
Hispanic American							
Hispanic-American							Lange *et al*. 2005
rural area		272				36	
urban area		448				45	
Mixed ancestry from Brazil: 51% Mulatto 35% Caucasian.		1576	21	47	32	56	Pereira *et al*. 2003
African							
African-American		335	23	51	26	52	Ellsworth *et al*. 2002
African-American		272				55	Lange *et al*. 2005
African-American		74	16	53	31		Lima *et al*. 2007
African-American ♀		105				44	Terra *et al*. 2005

Table 2.27: *continued*

Population	Weight and disease status	n	Arg/ Arg %	Arg/ Gly %	Gly/ Gly %	Gly16 allele %	References
Asian							
Japanese	obese	124	28	50	22	47	Yamada *et al*. 1999
	non-obese	450	25	50	25	50	
Japanese	obese	108	28	52	20	46	Ishiyama-Shigemoto *et al*.
	non-obese	400	25	50	25	50	1999
Japanese ♂	BMI>25	55	18	49	33	57	Kawaguchi *et al*. 2006
Japanese ♂	BMI>25 &						Kawaguchi *et al*. 2006
	BP ↑ over 5 yrs	27	0	41	59		
	No BP ↑ over 5 yrs	28	36	57	7		
Japanese ♂	BMI>25 &						Kawaguchi *et al*. 2006
	weight gain over 5 yrs	33	9	42	49		
	weight stable over 5 yrs	22	32	59	9		
Japanese ♂	All subjects BMI<25 +	219	31	47	22	46	Masuo *et al*. 2007
	normal NE	182	34	51	15	40	
	high NE	37	13	30	57	72	
Taiwanese	DM2	130	37	43	20	42	Chang *et al*. 2002
	Non-DM2	130	24	58	18	47	
Korean	Non-DM2 & non-obese	43	40	51	9	35	Kim *et al*. 2002
	Non-DM2 & obese	46	39	50	11	36	
Korean	DM2 & non-obese	44	34	39	27	40	Kim *et al*. 2002
	DM2 & obese	62	42	47	11	35	

BMI = body mass index, DM2 = Type 2 diabetes, BP = blood pressure, NE = norepinephrine.

2.7.6 Associations with obesity related phenotypes

Weight, BMI and obesity

The majority of studies summarized in Table 2.28 (10 out of 12 published studies) did not show an association between obesity and the *ADRB2* Arg16Gly polymorphism in Caucasians (six studies) and in subjects with African (two studies) and Asian (two studies) ancestry. A meta-analysis of 13 case-control studies including populations from different ethnicities revealed no association between obesity and the *ADRB2* Arg16Gly polymorphisms (Gjesing *et al*. 2007). Two studies have shown that obesity is associated with a lower frequency of the Gly16Gly homozygous genotype in Japanese obese women (Ishiyama-Shigemoto *et al*. 1999) and a lower frequency of Gly16-allele carriers in French Canadian men with a BMI more than 35 compared to controls (Ukkola *et al*. 2000). In line with these results, a lower BMI was found for Gly16Gly homozygotes in Caucasian females (Meirhaeghe *et al*. 2001) and a Brazilian cross-sectional sample consisting of mixed ethnicities (Perreira *et al*. 2003). However, in contrast to these findings other studies have reported an association between the Gly16Gly homozygous genotype or Gly16-allele carriers and a higher BMI in Caucasian (Ehrenborg *et al*. 2000) and Brazilian males (Mattevi *et al*. 2006) and with weight gain in males over a period of seven years (Van Rossum *et al*. 2002). Furthermore, in a longitudinal follow-up of different cohorts with subjects from birth to their late 30s an association between Gly16-allele carriers and a higher BMI was observed in Caucasian males and African-Americans (Ellsworth *et al*. 2002). This difference in BMI between the Arg16Gly genotypes became more pronounced with age and was not seen in females. It was found that the BMI of males who are Gly16-allele carriers increased at a greater rate over time than the Arg16Arg homozygotes. The association that was not significant when subjects were in childhood and adolescent years, tended to reach significance by 21 years of age, and reached significance at the age of 35 years (Ellsworth *et al*. 2002). This relates to a 4% higher BMI found in Gly16-allele carriers at age 26 and an 8% higher BMI at age 32 (Ellsworth *et al*. 2002). In contrast with males, females (when they were ages 12 and 21 years) who are Gly16 allele carriers had lower BMIs than Arg16Arg homozygotes (Ellsworth *et al*. 2002).

In non-Caucasian populations two studies reported an association between the Arg16Gly genotype and BMI. The Gly16Gly homozygous genotype was associated with a lower BMI in Koreans with type 2 diabetes (Kim *et al*. 2002). However, in Japanese Gly16-allele carriers had a significant increase in weight over a follow-up period of five years (Kawaguchi *et al*. 2006). Furthermore, three studies in subjects with African ancestry (Lange *et al*. 2005, Terra *et al*. 2005, Lima *et al*. 2007) and two on Japanese (Ishiyama-Shigemoto *et al*. 1999, Masuo *et al*. 2007) did not find any association.

In summary, at this stage most studies indicate no association between the *ADRB2* Arg16Gly polymorphism and BMI or obesity. Furthermore, the results of the positive association studies were equivocal with some linking the Gly16-allele carriers or Gly16Gly homozygotes with a higher BMI or with weight gain while others linked these genotypes with a lower BMI or obesity prevalence.

Table 2.28: The association between the *ADRB2* Arg16Gly polymorphism and weight, BMI and obesity.

Population	Weight and disease status	n	Study design	Association with obesity		Association with BMI		Reference
				Yes	No	Yes	No	
Caucasian								
Austrian ♀	Obese Non-obese	183 216	Case-control	-	✓	-	✓	Oberkofler *et al*. 2000
Danish		7808	Meta-analysis	-	✓	-	✓	Gjesing *et al*. 2007
Sweden healthy ♂	Non-obese	180	Population based	ni	ni	♂ Gly16Gly = ↑ BMI	-	Ehrenborg *et al*. 2000
Sweden ♂		284	Cross-sectional	-	✓	-	✓	Rosmond *et al*. 2000
Sweden ♀	Obese Non-obese	82 58	Cohort sample with case-control analysis	-	✓	-	✓	Large *et al*. 1997
Netherlands	Over 7 yrs: Weight gain Stable weight	 286 296	Cohort: follow-up of 7 years	ni	ni	♂ with 7 yr weight gain = ↑ Gly16Gly vs stable weight subjects	In ♀	Van Rossum *et al*. 2002
German children	Obese Lean	296 134	Case-control	-	✓	ni	ni	Tafel *et al*. 2004
UK		604	Population based cohort	ni	ni	♀ Arg16 = ↑ BMI	In ♂	Meirhaeghe *et al*. 2001
France		836	Population based	-	✓	-	✓	Meirhaeghe *et al*. 2000
French-Canadian		743	Family-based	BMI≥35 ♂ = ↓ Gly16-allele	-	ni	ni	Ukkola *et al*. 2000
American		161	Population based	ni	ni	-	✓	Lima *et al*. 2007
American ♀	>18 yrs referred for coronary angiogram for suspected CHD	538	Case-control	-	✓	-	✓	Terra *et al*. 2005
Mixed ancestry: African American & Caucasian American		1151	Longitudinal: 3 cohorts newborn-, childhood- & teenage each followed for 20 yrs	ni	ni	Childhood cohort, Gly16 ♂: ❑ 0.2% BMI ↑ pa ❑ = 4% ↑ BMI at age 26 Teenage cohort, Gly16 ♂: ❑ 0.4% BMI ↑ pa ❑ = 8% ↑ BMI at 32 yrs	in newborn /childhood cohorts In all ♀	Ellsworth *et al*. 2002
Hispanic American								
Brazilians		334	Population based	ni	ni	♂ Gly16 = ↑ BMI	In ♀	Mattevi *et al*. 2006
Mixed ancestry: African American & Hispanic American:		992	Cross-sectional	-	✓	-	✓	Lange *et al*. 2005
Mixed ancestry from Brazil: 51% Mulatto 35% Caucasian.		1576	Cross-sectional	ni	ni	Gly16Gly = ↓ BMI	-	Pereira *et al*. 2003

Table 2.28: *continued*

Population	Weight and disease status	n	Study design	Association with obesity		Association with BMI		Reference
				Yes	No	Yes	No	
African								
African American ♀	>18 yrs referred for coronary angiogram for suspected CHD	105	Case-control	-	✓	-	✓	Terra *et al*. 2005
African American		74	Population-based	ni	ni	-	✓	Lima *et al*. 2007
Asian								
Japanese	Obese Non-obese	108 400	Case-control	obese ♀ = ↓ Gly16Gly	in ♂	-	✓	Ishiyama-Shigemoto *et al*. 1999
Japanese	Obese Non-obese	124 450	Case-control	-	✓	ni	ni	Yamada *et al*. 1999
Japanese ♂	Obese + weight gain Obese + stable weight	33 22	Cohort: Follow-up for 5 years	ni	ni	Weight gain over 5 year = ↑ Gly16Gly% Gly16 allele = BMI ↑ over 5 years (NS for Arg16Arg)	-	Kawaguchi *et al*. 2006
Japanese ♂	Non-obese	219	Company-based sample: baseline and change over 5 years	ni	ni	-	✓	Masuo *et al*. 2007
Korean	Obese & Non-obese DM2 & non-DM2	195	Cohort	-	✓	DM2: Gly16Gly = ↓ BMI	-	Kim *et al*. 2002

*Recessive model = compares the CC homozygotes with G-allele carriers; Dominant model = compares GG homozygotes with C-allele carriers, childhood and adolescent years the Gly16 allele carriers generally gained 0.2% and 0.4% more per year respectively, which relates to a 4% higher BMI at age 26 and a 8% higher BMI at age 32

Measures of body fat distribution and content

In Caucasians, the majority of studies have reported no association between the *ADRB2* Arg16Gly polymorphism and body fat distribution or content (Table 2.29). The studies that did report a positive association indicated that the mutant Gly16-allele carriers had a higher waist circumference than subjects with the Arg16Arg genotype (Mattevi *et al*. 2006). In a mixed ethnicity sample consisting of African American and Caucasian males the Gly16-allele carriers experienced a 0.5% and 2% increase in subscapular skinfolds per annum in a childhood and teenage cohort respectively. Consequently, the Gly16-allele carriers had a 20% and 27% higher subscapular skinfold measurement in their 20s (Ellsworth *et al*. 2002). However, contradictory results were reported in a French population with Gly16-allele carriers having a lower waist circumference, hip circumference and waist-to-hip ratio (Meirhaeghe *et al*. 2000).

No positive associations were found by the studies that included subjects with Hispanic or African ancestry. In Japanese men, Gly16-allele carriers had a higher body fat mass, waist-hip-ratio, leptin, norepinephrine and higher five-year increases in body fat mass, waist-hip-ratio and leptin (Kawaguchi *et al*. 2006, Masuo *et al*. 2007). As only a limited number of studies measured these associations in populations with Hispanic, African and Asian ancestry, more research is necessary to confirm these findings.

Table 2.29: The association between the *ADRB2* Arg16Gly polymorphism and measures of body fat distribution and content.

Population	Associations	No associations	Reference
Caucasian			
French	Gly16 = ↓ WC, HC, WHR	-	Meirhaeghe *et al*. 2000
UK	-	WHR	Meirhaeghe *et al*. 2001
Danish		WC, HC, WHR	Gjesing *et al*. 2007
Sweden ♂	-	WHR, abdominal sagittal diameter	Rosmond *et al*. 2000
Sweden ♀	-	WHR, body FM, fat cell volume.	Large *et al*. 1997
American ♀	-	WHR, WC	Terra *et al*. 2005
American	-	body fat %	Lima *et al*. 2007
Mixed ancestry African-Americans and Caucasian-Americans	<u>Childhood cohort, Gly16 ♂:</u> ❑ 0.5% SS ↑ pa ❑ = 20% ↑ SS at 20 yrs <u>Teenage cohort, Gly16 ♂:</u> ❑ 2% SS ↑ pa ❑ = 27% ↑ SS at 22 yrs ❑ ↑ SS, TS	in newborn cohort or in ♀	Ellsworth *et al*. 2002
European Brazilians	♂ Gly16 = ↑ WC	WC in ♀	Mattevi *et al*. 2006
Hispanic American			
Mixed ancestry: African-American & Hispanic-American	-	WHR, VAT, SAT, VSR	Lange *et al*. 2005
Mixed ancestry from Brazil: 51% Mulatto 35% Caucasian.	-	WHR, WC	Pereira *et al*. 2003
African			
African-American	-	body fat %	Lima *et al*. 2007
African-American ♀	-	WHR, WC	Terra *et al*. 2005
Asian			
Japanese ♂ with BMI>25	Gly16 = ↑ body FM, WHR, leptin, ↑ in body FM, leptin over 5 yrs (NS for Arg16Arg)	NE	Kawaguchi *et al*. 2006
Japanese ♂ with BMI<25	↑ Gly16 = high p-NE Gly16 = ↑ baseline and 5 yrs body fat, WHR, p-NE Gly16=higher ↑ in body fat & WHR over 5 yrs	-	Masuo *et al*. 2007
Korean	-	WHR, body fat %	Kim *et al*. 2002

HC = hip circumference, WC = waist circumference, WHR = waist-hip-ratio, SS = subscapular skinfolds, TS = triceps skinfolds, NE= norepinephrine, NS = non-significant, SAT = subcutaneous adipose tissue, VAT = visceral adipose tissue, VSR = visceral to subcutaneous ratio.

2.7.7 Associations with health indicators

Indicators of glucose and insulin homeostasis, blood lipid profile and blood pressure

The studies showing a positive association between the *ADRB2* Arg16Gly polymorphism and glucose and insulin homeostasis points to the possibility that Gly16Gly homozygotes or Gly16-allele carriers with Caucasian (Gjesing *et al*. 2007, Meirhaeghe *et al*. 2000, Dallongeville *et al*. 2003, Meirhaeghe *et al*. 2001) or Asian (Chang *et al*. 2002) ancestry are protected from the development of MetS or type 2 diabetes (Table 2.30). In contrast, Caucasians with these genotypes had higher total cholesterol and LDL cholesterol levels (Ukkola *et al*. 2000, Meirhaeghe *et al*. 2001).

The blood pressure results summarized in Table 2.30 are equivocal. Recent reviews also concluded that the results from a large number of studies in various ethnic groups have shown that the association between the *ADRB2* Arg16Gly polymorphism and blood pressure are controversial and probably not important in hypertension development (Hahntow *et al*. 2006, Brodde *et al*. 2008). In the 32 studies summarized by Hahntow *et al*. (2006), hypertension was associated with the Arg16-allele in five studies and with the Gly16-allele in seven studies, while 20 studies found no association. Controversial results were also obtained from studies investigating the association between this *ADRB2* polymorphism and heart failure (Brodde *et al*. 2008). Brodde *et al*. (2008) concluded that the *ADRB2* Arg16Gln27 haplotype might predict poor outcome of heart failure, but that large prospective studies need to substantiate these findings.

In summary, Gly16-allele carriers seem to have a lower risk to develop Type-2 diabetes. However, results for the association between the *ARDB2* Arg16Gly polymorphism and blood lipid profile or blood pressure are equivocal and no final conclusions can be drawn.

Table 2.30: The association between the *ADRB2* Arg16Gly polymorphism and indicators of glucose and insulin homeostasis, blood lipid profile and blood pressure.

Population	Indicators of glucose and insulin homeostasis		Blood lipids and blood pressure (BP)		Reference
	Associations	No associations	Associations	No associations	
Caucasian					
Sweden: non-obese healthy ♂	-	glucose	-	TG, VLDL, TC, LDL, HDL, BP	Ehrenborg *et al*. 2000
Sweden ♂	-	glucose, insulin	Arg16Gly = ↑ SBP vs. Arg16Arg	TG, TC, HDL, LDL, DBP	Rosmond *et al*. 2000
Sweden ♀	-	glucose, insulin	ni	ni	Large *et al*. 1997
Danish	DM2 = ↓ Gly16Gly	Glucose, insulin, 2h glucose, insulin after OGTT	Gly16Gly = ↓ SBP	-	Gjesing *et al*. 2007
French	Gly16 ♂ = ↓ insulin	insulin in ♀, glucose	-	TG, TC	Meirhaeghe *et al*. 2000
French	Gly16Gly ♂ = ↓ MetS, high glucose prevalence (NCEP ATPIII criteria)	-	Gly16Gly ♂ = ↓ prevalence of low HDL (NCEP ATPIII criteria)	High TG, BP (NCEP ATP III)	Dallongeville *et al*. 2003
French-Canadian	ni	ni	Gly16Gly = ↑ TC, Gly16Gly ♂ = ↑ TC, LDL	HDL, TG	Ukkola *et al*. 2000
UK	Gly16Gly ♀ = ↓ 30 min insulin increment	Fasting insulin, glucose In ♂: 30 min insulin increment	Gly16 ♀ = ↑ fasting NEFA, at 30min, NEFA AUC Gly16Gly ♀ = ↓ TG	TC, HDL, LDL. In ♂: TG, NEFA (fasting, 30 min, AUC)	Meirhaeghe *et al*. 2001
Italians	-	DM2	ni	ni	Pinelli *et al*. 2006
American	-	glucose, insulin, HbA1c, HOMA-IR	ni	ni	Lima *et al*. 2007
Hispanic American					
Mixed ethnicity from Brazil: 51% Mulatto, 35% Caucasian	-	glucose	Gly16Gly = ↑ SBP	TC, TG, LDL	Pereira *et al*. 2003
African					
African-American	-	glucose, insulin, HbA1c, HOMA-IR	ni	ni	Lima *et al*. 2007
Asian					
Japanese	-	glucose tolerance DM2	-	TC, TG BP	Ishiyama-Shigemoto *et al*. 1999
Japanese ♂ with BMI>25	-	-	BP ↑ over 5 yrs = ↑ Gly16%	-	Kawaguchi *et al*. 2006
Japanese ♂ with BMI<25	-	HOMA-IR	-	BP	Masuo *et al*. 2007
Taiwanese	Gly16 = ↓ DM2 later onset of DM2	-	ni	ni	Chang *et al*. 2002
Korean		Glucose, insulin, DM2	-	LDL, HDL, TG, FFA, BP	Kim *et al*. 2002

ni = not investigated, MetS = Metabolic Syndrome, DM2 = Type 2 Diabetes Mellitus, TG = triglycerides, TC = Total Cholesterol, HOMA-IR = , HDL = High density lipoprotein, VLDL = Very low-density lipoprotein, LDL = Low density lipoprotein, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, IDF = International Diabetes Federation, NCEP ATPIII = National Cholesterol Education Program Adult Treatment Panel III, NEFA = non-esterified fatty acids, OGTT = oral glucose tolerance test, AUC = area under curve.

2.7.8 Associations with weight loss outcomes

Only one weight loss intervention that investigated the role of the Arg16Gly polymorphism and weight outcome was found (Table 2.31). Japanese men (n=154) were enrolled in a two year weight loss intervention consisting of individual one hour teaching and counselling sessions every week in the first month and then biweekly for the rest of the 24 months (Masuo *et al*. 2005). The main findings indicate that the mutant Gly16-allele carriers were resistant to weight loss or had a slower weight loss compared to the wild-type Arg16Arg homozygotes. Although significant decreases were reported in all genotype groups from baseline to 24-month follow-up, the Gly16-allele carriers had higher BMIs at 24 months and a higher body fat mass, waist-hip-ratio, norepinephrine and leptin levels at baseline and throughout the study period. This study points to the possibility that a weight loss intervention consisting of a high carbohydrate and low fat diet, exercise and nutrition education and counselling sessions is effective to reduce weight in Arg16Arg homozygotes (Masuo *et al*. 2005).

The effect of the *ADRB2* Arg16Gly polymorphism on changes in body fat content was measured in two independent studies (Garenc *et al*. 2003, Ukkola *et al*. 2003) using similar exercise programmes consisting of cycling sessions on ergometers three times a week for 20 weeks with an increasing intensity and duration protocol based on each subjects' heart rate. Ukkola *et al*. (2003) showed that Black subjects (n=205) who were Arg16-allele carriers experienced greater decreases in fat mass and abdominal total fat area. Similarly, white Arg16Arg homozygous women experienced greater reductions in BMI, fat mass and body fat percentage following the 20-week exercise programme (Garenc *et al*. 2003). In contrast, Garenc *et al*. (2003) found no association in Black subjects between the Arg16Gly polymorphism and changes in BMI, fat mass, body fat percentage, sum of eight skinfolds, abdominal visceral or subcutaneous or total abdominal fat following the exercise intervention. Furthermore, Ukkola *et al*. (2003) showed no association between the Arg16Gly polymorphism and body fat changes for white subjects (n=415), but they did notice a similar trend in Caucasians similar to that reported for Blacks (Ukkola *et al*. 2003) and Caucasian women (Garenc *et al*. 2003).

The results point to the possibility that mutant Gly16-allele carriers are resistant to weight loss or decreases in body fat content when following a 24-month weight loss intervention or an exercise regime. So far it has been shown that Japanese men with the wild-type Arg16Arg genotype benefit from following a 24-month weight loss intervention, while Black Arg16-allele carriers and White Arg16Arg women benefit from following an exercise regime. Whether these results are applicable to all Caucasian, African and Asian populations still needs to be investigated.

Table 2.31: The association between the *ADRB2* Arg16Gly polymorphism and change in weight (BMI) and other variables following a weight loss intervention.

Population	Sample size	Genotype frequencies	Inclusion criteria	Weight loss intervention	Variables measured	Results	Reference
Japanese ♂	154	Arg16Arg=27% Arg16Gly=49% Gly16Gly=23% Gln27Gln=81% Gln27Glu=19% Glu27Glu=0%	BMI≥25 and BMI<30	***24 months intervention*** Private teaching & counselling sessions: ❑ Month 1 = 1 session/ week ❑ Next 23 months: 1 session biweekly. Diet =1600kcal/d, 15% fat, 55% carbohydrate, 30% protein. Exercise = >1h / day (recorded with step counters)	BMI, total body FM, WHR, BP, heart rate, NE, Leptin, HOMA-IR	Subjects divided in 4 groups: 1) weight loss maintenance group, 2) rebound weight loss group, 3) slow weight loss group, 4) weight loss resistance group Weight loss group = ↓ Gly16-allele frequency Weight loss group & rebound group = ↓ Glu27 frequency Compared Arg16Arg vs. Gly16 allele carriers at baseline, 6 and 12 months. From baseline to 24 months both groups had significant ↓ in BMI, total body FM, WHR, DBP, NE, Leptin HOMA-IR. However, Gly16 allele carriers had: ❑ ↑ BMI at 24 months. ❑ ↑ total body FM at baseline, 6 and 24 months ❑ ↑ WHR at baseline and 24 months. ❑ ↑ NE at baseline, 6 and 12 months ❑ ↑ Leptin at baseline, 6 and 12 months ❑ ↑ HOMA-IR at baseline. Compared Gln27Gln vs. Glu27 allele carriers at baseline, 6 and 12 months. From baseline to 24 months both groups had significant ↓ in BMI, total body FM, WHR, DBP, NE, Leptin. However, Glu27 allele carriers had: ❑ ↑ total body FM at baseline, 6 and 24 months. ❑ ↑ NE at baseline, 6 and 12 months ❑ ↑ Leptin at baseline, 6 and 12 months	Masuo *et al*. 2005

BMI = body mass index, WHR = waist-hip ratio, FM = fat mass, BP = blood pressure, NE = norepinephrine

2.8 G-protein β_3 subunit gene (*GNB3*)

2.8.1 The *GNB3* gene

The human *GNB3* gene was first cloned in 1990 (Levine *et al*. 1990) and located to the short arm of chromosome 12 (12p13) using somatic cell hybrids and *in situ* hybridization (NCBI, 2009; Modi *et al*. 1989, Levine *et al*. 1990). The *GNB3* gene consists of 7183 bp and 11 exons (NCBI, 2009).

2.8.2 The GNB3 protein

The *GNB3* gene encodes a β-subunit of the guanine nucleotide binding proteins (G-proteins) comprising of 340 amino acids. The G-proteins are heterotrimers consisting of three subunits namely the Gα-, Gβ-, and Gγ-subunits. In humans 21 Gα-subunits encoded by 17 genes, six Gβ-subunits encoded by five genes (including the β3-subunit or *GNB3*) and 12 Gγ subunits have been identified (Milligan & Kostenis 2006, Oldham & Hamm 2006).

The amino acid sequences of the six Gβ-subunits share a 50 to 90% homology (Oldham & Hamm 2006). The structure of the Gβ-subunits consist of seven bladed β-propellers (Figure 2.6) that are protein folds formed from a common structural motif, the WD 40 (tryptophan-aspartic acid) repeat domains on the genes encoding the Gβ-proteins (Li & Roberts 2001, Milligan & Kostenis 2006, Oldham & Hamm 2006). Each blade has four antiparallel β-strands with the WD 40 sequence forming the last three strands of a blade and the first strand of the next blade. The final blade requires the first few sequences of the N-terminus to close the ring structure, while the remaining sequences on the N-terminus forms a α-helical coil that is essential for binding to the Gγ-subunits.

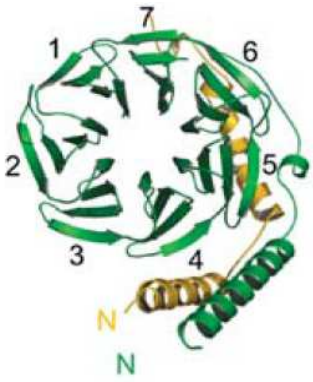

Figure 2.6: The seven bladed β-propeller structure of the G-proteins (from Oldham & Hamm 2006).

The Gβ- and Gγ-subunits cannot be expressed independently and exist as a functional dimer that is only dissociated from each other by denaturation (Jones *et al*. 2004, Oldham & Hamm 2006). Most of the six Gβ-subunits can interact with most of the 12 Gγ-subunits. However not all 60 possible combinations of dimers form

and selectivity between specific Gβ- and Gγ-subunits has been identified (Oldham & Hamm 2006). The Gβγ-dimer binds to the Gα-subunit with high affinity to form the heterotrimeric αβγ G-protein and dissociates from the Gα-subunit upon activation. The conformation of the Gβγ-dimers remains constant whether it is in the heterotrimeric complex or in the free active dimer state (Cabrera-Vera *et al*. 2003). However, distinct conformational changes are prevalent in the Gα-subunit when it is bound to the Gβγ-dimer as an inactive heterotrimer or when it exists as an activated Gα-subunit (Jones *et al*. 2004, Milligan & Kostenis 2006). The G-proteins are principally localized to the inner side of plasma membranes and expressed in all cells of the human body (Siffert 2005).

2.8.3 Physiological function of GNB3

The G-proteins are the most commonly used signal transducers in eukaryotic cells, allowing extracellular stimuli to induce signals across cell membranes and translating these into a cellular response, affecting neurotransmission, growth, differentiation or cell death (Siffert 2005, Koelle 2006). An interaction between the G-proteins and a guanine nucleotide exchange factor (GEF), of which the most common are the members of the GPCRs on cell membranes, is necessary for activation of the G-proteins and consequent signal transduction (Jones *et al*. 2004, Milligan & Kostenis 2006).

In the basal state, the Gα-subunit is attached to GDP while the Gβγ-dimer is anchored to the inner side of the plasma membrane and associated to the GDP-bound Gα-subunit to form an inactive αβγ heterotrimer (Jones *et al*. 2004, Penn & Benovic 2008). The binding of ligands such as hormones, neurotransmitters, chemokines, light and odorants to GPCRs facilitates coupling with the G-proteins. All three subunits of the G-proteins are required for successful coupling to GPCRs that result in conformational changes in GPCRs, increasing the affinity thereof for the ligand. The activated GPCRs act as a GEF and consequently induce a structural change to the Gα-subunit, resulting in the release of GDP and the binding of GTP to the Gα-subunit. This binding destabilizes the heterotrimer, followed by the dissociation of the activated G-protein from GPCRs and the formation of a Gα-subunit-GTP complex and a Gβγ complex. Both these complexes can then activate or inhibit several downstream effector proteins such as adenylyl cyclases, phospholipase Cb (PLCb), tyrosine kinases, phosphodiesterases, phosphoinositide 3-kinase, GPCR kinases, ion channels, and molecules of the mitogen-activated protein kinase pathway, resulting in the initiation of signal transduction cascades and a variety of cellular functions. To terminate these effector activation cellular processes, the intrinsic GTPase activity of the Gα-subunit hydrolyses GTP to GDP. The αβγ subunits will then re-associate to form the inactive GDP bound Gαβγ heterotrimer to complete the cycle of signal transductions. The intrinsic GTPase activity of the Gα-subunit can be accelerated by RGS (regulators of G-protein signalling) proteins, which consequently modulate the duration of signalling events (Cabrera-Vera *et al*. 2003, Jones *et al*. 2004, Milligan & Kostenis 2006, Oldham & Hamm 2006, Bagos *et al*. 2007, Penn & Benovic 2008, Vogler *et al*. 2008).

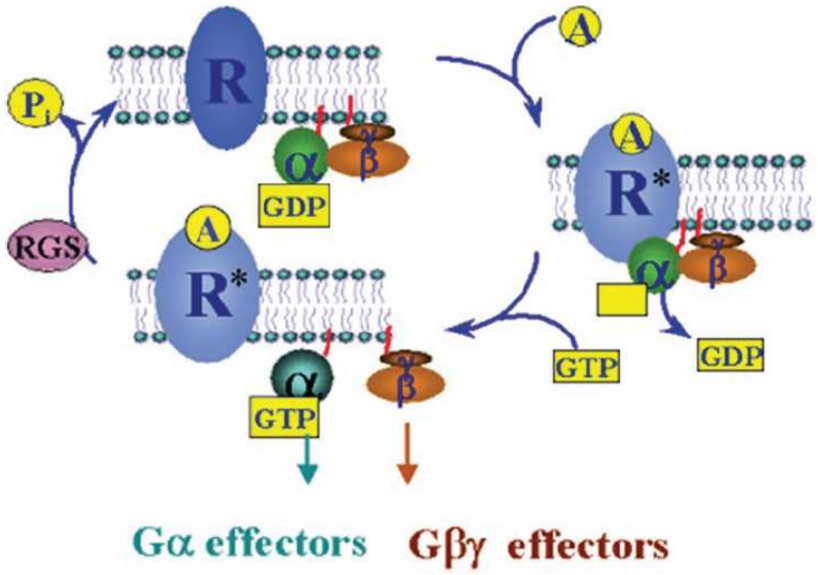

Figure 2.7: The activation of G-proteins through the binding of a ligand to GPCRs (from Cabrera-Vera *et al*. 2003).

The G-proteins are typically divided into four different subfamilies based on sequence homology, with each subfamily activating or inhibiting specific downstream effector proteins (Riobo & Manning 2005, Oldham & Hamm 2006, Penn & Benovic 2008):

- G_s-proteins (including G_s and G_{olf}) activate adenylyl cyclase,
- $G_{i/o}$-proteins (including G_{i1}, G_{i2}, G_{i3}, G_{o1}, G_{o2}, G_z, G_{t1}, G_{t2}, and G_{gust}) inhibit adenylyl cyclase,
- $G_{q/11}$-proteins (including G_q, G_{11}, G_{14}, and $G_{15/16}$) activate phospholipase C (PLC),
- $G_{12/13}$-protein (including G_{12} and G_{13}) activate Rho guanine nucleotide exchange factors (RhoGEFs), Na^+ H^+ exchangers, and PLC-ε.

The G-proteins are also located on the cell membranes of WAT where they interact with GPCRs and the adrenergic receptor, transducing signals that affect lipolysis in the adipocytes as described in Section 2.6.3, p87.

2.8.4 The *GNB3* C825T polymorphism

A synonoumous polymorphism in exon 10 of the *GNB3* gene results in a C to T transition at nucleotide position 825 without causing a change in Serine amino acid at codon 275 (Ser275Ser) of this gene (Siffert *et al*. 1998). Although this polymorphism does not affect the amino acid sequence, the mutant 825T allele causes the generation of *GNB3* splice variants (Siffert 2005). Siffert *et al*. (1998) identified a truncated *GNB3* splice variant (*GNB3s*) in which nucleotides 498 to 620 of exon 9 are deleted due to alternative splicing. This in-frame deletion is associated with the 825T allele and results in a variant GNB3 protein lacking 41 amino acids and one of its seven WD repeat domains (Siffert *et al*. 1998). Another *GNB3* splice variant, designated *GNB3s-2*, is formed when alternative splicing causes the deletion of 129 bp in the coding sequence of the wild-type GNB3 protein. The expression of *GNB3s-2* mRNA in the heart, blood cells and tumor tissue correlates with the 825T allele

(Rosskopf *et al*. 2003a). Both these truncated *GNB3* splice variants result in the expression of a protein that now consists of a six-bladed β-propeller structure instead of the seven-bladed wild-type GNB3 protein (Siffert *et al*. 1998, Rosskopf *et al*. 2003a). A third splice variant, *GNB3v*, is generated when alternative splicing of intron 9 causes the formation of a new exon 10a and consequently the GNB3v protein is expressed with four WD repeat domains and a novel C terminus (Rosskopf *et al*. 2003b). However, *GNB3v* was not found to be associated with an allele of the *GNB3* C825T polymorphism, and therefore it was suggested that *GNB3v* does not contribute to the phenotypes observed in association with the 825T-allele (Rosskopf *et al*. 2003b).

The *GNB3* splice variants have been associated with enhanced activation of the G-proteins, which ultimately increases G-protein signalling (Siffert *et al*. 1998, Rosskopf *et al*. 2003a, Rosskopf *et al*. 2003b). This was observed in COS-7 cells and Sf9 insect cells in which the over-expression of the *GNB3s* variant resulted in enhanced GTPγS binding (Siffert *et al*. 1998) and increased chemotaxis (Virchow *et al*. 1999) respectively. It was also shown that the *GNB3s* variant could form dimers with various other Gγ-subunits than the wild-type GNB3 (Rosskopf *et al*. 2003a). Furthermore, *ex vivo* studies showed that humans who are 825T-allele carriers exibited enhanced features of signal transduction such as enhanced chemokine-stimulated chemotaxis and proliferation in neutrophils and lymphocytes (Virchow *et al*. 1998, Lindemann *et al*. 2001) and enhanced epinephrine-induced platelet aggregation (Naber *et al*. 2000). It was further indicated that the 825T-allele is associated with altered modulation of acetylcholin-activated K^+ channels in atrial myocytes (Dobrev *et al*. 2000). Siffert (2005) concludes that sufficient studies support the fact that the 825T-allele is a valid genetic marker associated with increased G-protein signal transduction.

The exact physiological role of the *GNB3* 825T variant in the development of obesity is not fully understood. It has been suggested that the predisposition to obesity may result from the observed decreased catecholamine induced lipolysis in adipose tissue of the 825T variant *GNB3* (Hauner *et al*. 2002, Rydén *et al*. 2002). Rydén *et al*. (2002) investigated the influence of the *GNB3* C825T polymorphism on lipolysis in isolated subcutaneous fat cells from 114 obese subjects. These authors showed that TT homozygous subjects had lower circulating free fatty acids and glycerol and decreased β-adrenoceptor agonist-stimulated lipolysis. More specifically, it was found that norepinephrine induced lipolysis was reduced in adipocytes of *GNB3* TT homozygotes (Rydén *et al*. 2002). This finding was supported by Hauner *et al*. (2002) who showed decreased catecholamine induced lipolysis in the adipose tissue collected from ten women who are T-allele carriers compared to ten CC homozygotes. The C825T polymorphism was not associated with adipocyte differentiation in the adipose tissue collected from a larger sample of 65 women (Hauner *et al*. 2002). Although these findings are not in line with increased signal transduction associated with the *GNB3* splice variants that would be expected to result in increased lipolysis, Ruiz-Velasco and Ikedal (2003) hypothesized that obesity and other resultant phenotypes may be due to a lack of the functional wild-type GNB3 protein rather than increased activation of the mutated GNB3 protein. This is supported by the finding of Rydén *et al*. (2002) that the GNB3 protein content in adipocytes of TT homozygous subjects was markedly reduced. However, more research is required to elucidate the physiological function of the *GNB3* splice variants and how this contributes to the development of obesity (Vogler *et al*. 2008).

2.8.5 Genotype and allele frequencies

The wild-type CC genotype is almost absent in populations from African descent, but found at a frequency in the range of 22 to 29 % in Asian populations (Table 2.32). In most Caucasian populations the CC and CT frequencies are found in similar ranges around 45%. The lowest frequency of the mutant TT genotype is found in Caucasian populations (around 10%), while populations with African ancestry show the highest frequency (around 60%), with Asian populations having an intermediate frequency (around 20%). Consequently, the lowest 825T-allele frequency is found in Caucasians, while an intermediate frequency is reported for Asians and the highest frequency are found in African descendants.

Table 2.32: Genotype frequencies of the *GNB3* C825T polymorphism and the frequency of the mutant T-allele.

Population	Weight or disease status	n	CC %	CT %	TT %	T-allele	References
Caucasian							
Caucasians*		3289	45	44	11	33	Siffert *et al*. 1999a
Belgium		1512	47	44	9	31	Brand *et al*. 2003
Italy		1359	45	43	12	33	Casiglia *et al*. 2008
		794	49	42	9		Gutersohn *et al*. 2000
German	HT	197	49	41	10	31	Siffert *et al*. 1999b
	Lean HT sub-sample		60	32	8	24	
	Overweight HT sub-sample		45	46	8	31	
	Obese HT sub-sample		40	40	20	10	
German: mothers		181	41	49	10		Hocher *et al*. 2000
babies		113	45	41	14		
German ♂		277	46	44	10	32	Siffert *et al*. 1999a
German		774	45	45	10	32	Stefan *et al*. 2004
German	Controls	733	49	41	10		Renner *et al*. 2007
	CAD pt all	2583	50	41	9		
	CAD with MI	1370	48	42	10		
Spain	Essential hypertensive	130	45	47	8		Poch *et al*. 2002
Spain	Essential hypertensive	76	33	55	12		Martín *et al*. 2005
	Normotensive	78	37	46	17		
Austrian		932	47	45	8	31	Wascher *et al*. 2003
Scandinavian	obese	114	54	39	7		Rydén *et al*. 2002
Danes	Glucose-tolerant	4723	49	41	10	30	Andersen *et al*. 2006
Danes	DM2	1358	46	46	9	32	Andersen *et al*. 2006
Danes	Normotensive	4193	48	42	10	31	Andersen *et al*. 2006
Danes	Hypertensive	3139	49	41	10	30	Andersen *et al*. 2006
Italian	Grade I HT	461	48	44	8		Sartori *et al*. 2003
Brazilian Caucasian		548	42	44	14	36	Danoviz *et al*. 2006
Australian	Obese	92	52	40	8	28	Benjafield *et al*. 2001
	Non-obese	189	53	43	3	0.25	
Australian	BMI>25 & normotensive	84	56	42	2	27	Benjafield *et al*. 2001
	BMI≤25 & normotensive	105	50	45	5	23	
Australian	BMI>25 & HT	55	16	69	15	49	Benjafield *et al*. 2001
	BMI≤25 & HT	56	30	63	7	38	
Americans		10988	48	43	9	31	Grove *et al*. 2007
Americans ♀	Suspected CHD	485	54	40	6	27	Terra *et al*. 2005
Hispanic American							
Mexican	HT	180	41	46	13	36	Kopf et al. 2008

Table 2.32: *continued*

Population	Weight or disease status	n	CC %	CT %	TT %	T-allele	References
Native American							
Canadian Nunavut Inuit		213	22	54	24	50	Hegele *et al*. 1999
Brazilian Mulatto		795	26	51	23	51	Danoviz *et al*. 2006
Canadian Oji-Cree		447	21	58	21	50	Hegele *et al*. 1998
Amerindians*		245	53	40	7	26	Siffert *et al*. 1999a
African							
Black Africans*		1015	4	34	62	79	Siffert *et al*. 1999a
African-Americans		175	6	38	56		Poston *et al*. 2002
African-Americans		3728	7	38	54	73	Grove *et al*. 2007
African-Americans ♀	Suspected CHD	94	7	29	64	79	Terra *et al*. 2005
South African Black ♂		254	2	28	70		Siffert *et al*. 1999a
Namibian Bushmen		97	9	47	41	66	Siffert *et al*. 1999a
Zimbabwean Black ♂		459	3	30	67		Siffert *et al*. 1999a
West Africans & Caribbean's		428	4	34	62	79	Dong *et al*. 1999
West Africans		158	3	30	67		Dong *et al*. 1999
Caribbean's		270	5	35	60		Dong *et al*. 1999
Brazilian Africans		122	14	47	39	37	Danoviz *et al*. 2006
Asian							
Japanese		806	29	45	27	49	Yamamoto *et al*. 2004
Japanese	DM2	427	29	46	25	48	Hayakawa *et al*. 2007
	Random healthy sample	368	23	49	28	47	
Japanese	Extremely obese (BMI>30)	208	28	48	24	48	Ohshiro *et al*. 2001
	Controls	150	25	49	26	51	
Japanese ♂	Normal weight	1131	25	52	23	49	Suwazono *et al*. 2004
	Overweight	322	25	50	25	50	
Japanese ♀	Normal weight	989	25	55	20	48	Suwazono *et al*. 2004
	Overweight	183	28	50	22	47	
Chinese	HT	585	23	50	27	52	Huang *et al*. 2003
	Controls	580	22	52	26	52	
Korean ♂	Obese (BMI>25)	152	26	57	17	45	Lee *et al*. 2005b
	Non-obese (BMI≤25)	130	25	52	23	49	
Chinese	EH	290	22	50	28	47	Wang *et al*. 2005
	NT	244	24	49	27	48	
Chinese ♂		960	27	51	22	48	Siffert *et al*. 1999a
Asian*		520	28	53	19	46	Siffert *et al*. 1999a

*Includes populations from various countries, HT = hypertension, CAD = coronary artery disease, MI = myocardial infarction, DM2 = Type 2 diabetes mellitus, CHD = coronary heart disease, EH = essential hypertension, NT = normotension, BMI = body mass index.

2.8.6 Associations with obesity related phenotypes

Weight, BMI and obesity

Studies that have found a positive association between the *GNB3* C825T polymorphism and weight status (Table 2.33) indicated that the 825T-allele or TT genotype are associated with post-pregnancy weight retention (Gutersohn *et al*. 2000) and a higher obesity prevalence or risk for obesity development in Caucasians (Siffert *et al*. 1999a, Siffert *et al*. 1999b, Brand *et al*. 2003). Various studies have also reported a higher BMI for the TT

homozygous genotype or T-allele carriers (Siffert *et al*. 1999a, Siffert *et al*. 1999b, Benjafield *et al*. 2001, Poch *et al*. 2002, Stefan *et al*. 2004, Casiglia *et al*. 2008). However, eight of the 17 studies performed in Caucasians could not replicate these associations. A recent meta-analysis including 18903 subjects from 18 studies (of which 12 studies were performed in Caucasians, three in Asians, one in Africans and two in subjects with mixed ancestry) published from 1999 to 2007, found a non-significant trend (p=0.053) towards a higher BMI in TT homozygotes (Souza *et al*. 2008).

Only one of the six studies in populations with African ancestry summarized in Table 2.34 found an association between the 825T allele and a higher overweight (BMI≥25) and obesity (defined as BMI>27) prevalence in a South African Black population (Siffert *et al*. 1999a). These authors also proposed that the risk of obesity in Blacks is only increased when an obesogenic environment and the mutant 825T allele of *GNB3* co-exist. By comparing the BMIs of rural Black Zimbabweans and urban Black Zimbabweans and South Africans, Siffert *et al*. (1999a) found that the frequencies of the 825T allele were similar in the rural and urban Blacks. However the overweight and obesity prevalence was lower in the rural Blacks who follow a traditional low fat, high maize meal and vegetable diet and have high physical activity levels.

For Asian populations the majority of studies (seven out of eight) found no association between the *GNB3* C825T polymorphism and obesity or BMI.

In summary, the mutant 825T-allele may predispose Caucasian populations to weight gain and obesity. However, the *GNB3* C825T polymorphism does not seem to influence the weight of individuals with African or Asian ancestry.

Table 2.33: The association between the *GNB3* C825T polymorphism and weight, BMI and obesity.

Population	Weight or disease status	n	Study design	Associations with obesity		Associations with BMI		Reference
				Yes	No	Yes	No	
Caucasian								
Belgium		1512	Population based	T-allele ♂= ↑ obesity TT ♂= ↑ obesity	-	ni	ni	Brand *et al*. 2003
Italian	fertile ♀	173	Population based	ni	ni	TT = ↑ BMI	-	Casiglia *et al*. 2008
Italian	menopausal ♀	575	Population based	ni	ni	-	✓	Casiglia *et al*. 2008
Italian	fertile vs. menopausal ♀	748	Population based	ni	ni	CC menopausal ♀ = ↑ BMI vs. CC fertile ♀	between TT or TC ♀	Casiglia *et al*. 2008
	Nulliparous and primiparous ♀	794	Cross-sectional	Primiparous TT = ↑ overweight	in nulliparous	Primiparous TT + low PA = ↑ BMI	in nulliparous with low/ high PA in primiparous with high PA	Gutersohn *et al*. 2000
Austrian		932	Population-based	-	✓	-	✓	Wascher *et al*. 2003
German		774	Population based	ni	ni	TT+TC = ↑ BMI	-	Stefan *et al*. 2004
German ♂		277	Population based	obesity = ↑ TT & T-allele	-	TT = ↑ BMI TT+TC = ↑ BMI	-	Siffert *et al*. 1999a
German	HT pt	197		TT = ↑ obesity	-	TT = ↑ BMI	-	Siffert *et al*. 1999b
German	controls CAD pt	733 2583	Cross-sectional	ni	ni	-	✓	Renner *et al*. 2007
German: mothers babies		181 113		ni	ni	TC moms had babies with ↓ birthweight	-	Hocher *et al*. 2000
Danes	glucose-tolerant	4387	Population based	-	✓	-	✓	Andersen *et al*. 2006
Spain		130		ni	ni	TT+TC = ↑ BMI	-	Poch *et al*. 2002
Spain	HT	76	Hypertensive pt	ni	ni	-	✓	Martín *et al*. 2005
Scandinavian	obese	113		ni	ni	-	✓	Rydén *et al*. 2002
American ♀		485	Suspected CHD	-	✓	-	✓	Terra *et al*. 2005
Americans		10988	Population-based	-	✓	ni	ni	Grove *et al*. 2007
Australian:	obese non-obese	92 189	Case-control	-	✓	T carriers = ↑ BMI	-	Benjafield *et al*. 2001
Australian:	normotensive BMI>25 normotensive BMI≤25	84 105	Case-control	-	✓	-	✓	Benjafield *et al*. 2001
Australian:	hypertensive BMI>25 hypertensive BMI≤25	55 56	Case-control	-	✓	-	✓	Benjafield *et al*. 2001

Table 2.33: *continued*

Population	Weight or disease status	n	Study design	Associations with obesity		Associations with BMI		Reference
				Yes	No	Yes	No	
Hispanic American								
Mixed Brazilian population: African, Caucasian, Mulatto		1568	Cross-sectional	-	✓	-	✓	Danoviz *et al*. 2006
African								
West-Africans + Carribeans		428	Population based	ni	ni	-	✓	Dong *et al*. 1999
RSA Blacks		254	Population based	T allele = ↑ % BMI>25	-	ni	ni	Siffert *et al*. 1999a
Zimbabwean Blacks		459	Population based	-	✓	ni	ni	Siffert *et al*. 1999a
African-American ♀		94	Suspected CHD	-	✓	-	✓	Terra *et al*. 2005
African-Americans		3728	Population based	-	✓	ni	ni	Grove *et al*. 2007
African-Americans		175	Randomly selected	ni	ni	-	✓	Poston *et al*. 2002
Native American								
Nunavut Inuit		213	Cross-sectional	-	-	TT = ↑ BMI	-	Hegele *et al*. 1999
Canadian Oji-Cree		447	Population-based	ni	ni	-	✓	Hegele *et al*. 1998
Asian								
Japanese		806	Cross-sectional?	-	✓	-	✓	Yamamoto *et al*. 2004
Japanese	DM	427		ni	ni	-	✓	Hayakawa *et al*. 2007
Japanese		368		ni	ni	-	✓	Hayakawa *et al*. 2007
Japanese:	overweight & normal	2625	Case-control	-	✓	ni	ni	Suwazono *et al*. 2004
Japanese:	obese & non-obese	358	Case-control	-	✓	-	✓	Ohshiro *et al*. 2001
Chinese	HT & controls	1165	Case-control	ni	ni	-	✓	Huang *et al*. 2003
Korean:	obese & non-obese	282	Case-control	-	✓	-	✓	Lee *et al*. 2005b
China:		534	Case-control	ni	ni	-	✓	Wang *et al*. 2005
Chinese		960	Population based	T allele = ↑ % BMI>25	-	ni	ni	Siffert *et al*. 1999a

BMI = body mass index, DM2 = Type 2 diabetes mellitus, ♀ = female, ♂ = male, CHD = coronary heart disease, NGT = normal glucose tolerance, HT = hypertension

Measures of body fat distribution and content

Studies investigating the association between the *GNB3* C825T polymorphism and body fat distribution or body fat content were mostly performed on Caucasians (Table 2.34). The TT homozygous genotype was found to be associated with higher triceps skinfolds in Belgian males (Brand *et al*. 2003) and Italians and a higher body fat percentage in Germans (Stefan *et al*. 2004). Furthermore, pre-menopausal Italian TT homozygous females had higher triceps, supra-iliac and subscapular skinfolds and mean skinfold thickness compared to C-allele carriers. However, no differences in skinfold measurements were found between the genotype groups of menopausal females (Casiglia *et al*. 2008). When the skinfold measurements and genotypes of pre-menopausal Italian and menopausal women were compared, the only differences were observed between the CC homozygous group, while all women in the other genotype groups had similar measurements. The CC menopausal women showed a higher prevalence of truncal obesity and higher skinfold thickness compared to CC pre-menopausal women (Casiglia *et al*. 2008). Therefore, although the CC homozygous women seem to be protected from a higher body fat content when younger, they might be at risk for gaining body fat after menopause, explaining the disappearance of the difference in fat content between genotype groups. However, TT homozygous women are at risk for higher fat content already during their pre-menopausal years, which remains high in the TT menopausal women. It might therefore be important to target TT homozygous women for weight management interventions during their younger years.

A number of studies have not found any relationship between the *GNB3* C825T polymorphism and waist circumference or weight-to-hip ratio in Caucasians (Stefan *et al*. 2003, Wascher *et al*. 2003, Terra *et al*. 2005, Andersen *et al*. 2006, Danoviz *et al*. 2006, Kopf *et al*. 2008), African-Americans (Terra *et al*. 2005), Japanese with Type 2 diabetes (Hayakawa *et al*. 2007) and Koreans (Lee *et al*. 2005b).

Table 2.34: The association between the *GNB3* C825T polymorphism and measures of body fat distribution and content.

Population	Associations	No associations	Reference
Caucasian			
Belgium	TT ♂ = ↑ Triceps SF	In ♀	Brand *et al.* 2003
Italians	TT = ↑ triceps SF, MST	subscapular, suprailiac SF	Casiglia *et al*. 2008
Italian fertile ♀	TT = ↑ MST, triceps suprailiac, subscapular SF	-	Casiglia *et al*. 2008
Italian menopausal ♀	-	MST, subscapular, triceps, suprailiac SF	Casiglia *et al*. 2008
Italian fertile vs. menopausal ♀	CC menopausal ♀ = ↑ truncal obesity %, MST, subscapular, triceps, suprailiac SF	-	Casiglia *et al*. 2008
Austrian	-	WHR	Wascher *et al*. 2003
German	T carriers = ↑ body fat %	WHR	Stefan *et al*. 2004
Danes glucose tolerant	-	WHR	Andersen *et al*. 2006
American ♀	-	WHR, WC	Terra *et al*. 2005
Hispanic American			
Mixed Brazilian‡	-	WHR	Danoviz *et al*. 2006
Mexicans	-	WHR	Kopf *et al*. 2008
African			
African-American	-	WHR, WC	Terra *et al*. 2005
Native American			
Canadian Nunavut Inuit	TT = ↑ WC, HC, triceps, subscapular SF	WHR	Hegele *et al*. 1999
Asian			
Japanese DM2 pt	-	WC, VFA, SFA	Hayakawa *et al*. 2007
Koreans	-	Body fat %, WC, WHR	Lee *et al*. 2005b

‡ Consisting of Caucasian, African and Mulatto ancestry, WC = waist circumference, WHR = waist-to-hip ratio, MST = mean skinfold thickness, SF = skinfold.

2.8.7 Associations with health indicators

Indicators of glucose and insulin homeostasis

Associations were reported between the TT homozygous genotype and higher fasting glucose and insulin levels, HbA1c, insulin resistance and Type 2 diabetes prevalence in Caucasian populations (Poch *et al*. 2002, Brand *et al*. 2003, Wascher *et al*. 2003, Andersen *et al*. 2006). However, in contrast one report indicated an association in the opposite direction with TT homozygous Mexicans having a better glucose homeostasis profile (Kopf *et al*. 2008). In Asians, the *GNB3* C825T polymorphism does not seem to influence glucose homeostasis (Table 2.35).

Blood lipid profile and blood pressure

The role of the *GNB3* C825T polymorphism on hypertension pathogenesis and its association with hypertension prevalence and blood pressure measurements have been investigated intensively over the years (Bagos *et al*. 2007). Although Table 2.35 does not provide a complete summary of all the association studies published, it does point to the fact that discrepancy in results exists. Siffert (2005) pointed to study design limitations that contribute to the controversy in the literature. The latter review concluded that the majority of studies

confirmed a positive association between the 825T-allele carriers and an increased risk for hypertension in Caucasians, but that results for Asian populations were still controversial (Siffert 2005). This view was supported by a meta-analysis of 34 papers published before the end of February 2006 on the association between the *GNB3* gene and hypertension, including 14094 hypertensive cases and 17760 controls (Bagos *et al*. 2007). These authors confirmed that the TT homozygous genotype is associated with a higher risk for hypertension in Caucasians but not in Asian populations (Bagos *et al*. 2007).

From Table 2.35 it is clear that the *GNB3* C825T polymorphism may not influence blood lipid profile as no positive associations were reported. According to the review by Siffert (2005) a few studies did report positive associations between the *GNB3* C825T polymorphism and atherosclerosis as well as its main consequences, namely stroke and myocardial infarction. It was found that the 825T allele carriers had increased cardiac stroke volume, arterial stiffness, radial artery hypertrophy, carotid artery plaque causing atherosclerosis, stroke risk, unstable angina, severity of coronary artery disease, risk for three-vessel disease and the constriction of coronary arteries in response to pharmacological stimulation (Siffert 2005). Although some have indicated an association with increased MI, other studies did not repeat these findings (Siffert 2005).

In summary, the *GNB3* C825T polymorphism does not seem to influence blood lipid profile, but possibly CVD risk. However, the TT homozygous Caucasians have a higher risk for hypertension, secondary to obesity, while these associations are still contradictory in Asian populations.

Table 2.35: The association between the *GNB3* C825T polymorphism and indicators of glucose and insulin homeostasis, blood lipid profile and blood pressure.

Population	Glucose and insulin homeostasis		Blood lipids and blood pressure (BP)		Reference
	Associations	No associations	Associations	No associations	
Caucasian populations					
Belgium ♂	TT = ↑ insulin, HOMA-IR	glucose	TT = ↑ BP	-	Brand *et al*. 2003
Belgium ♀	-	glucose, insulin, HOMA-IR	-	BP	Brand *et al*. 2003
Italian ♀	-	DM2	-	HT	Casiglia *et al*. 2008
Italian fertile ♀	-	glucose, DM2, insulin, HOMA-IR	TT = ↑ SBP	TC, HDL, TG, DBP	Casiglia *et al*. 2008
Italian menopausal ♀	-	glucose, DM2, insulin, HOMA-IR	-	TC, HDL, TG, BP	Casiglia *et al*. 2008
Spain HT pt	-	glucose	-	BP, TG, TC	Martín *et al*. 2005
Spain: hypertensive	TT+TC =↑ insulin, 2h glucose, HbA1c and ↓ ISI	glucose	-	TC, TG, LDL, HDL	Poch *et al*. 2002
Austrian	T carriers: all = ↑ glucose, insulin ♂ & WHR>0.9 = ↓ insulin sensitivity (not in ♀)	DM2	-	TC, TG, HDL, LDL, BP, HT	Wascher *et al*. 2003
German CAD pt & controls	ni	ni	-	BP, CAD, MI, HT,	Renner *et al*. 2007
German HT pt	ni	ni	-	MI, stroke, all CVE	Siffert *et al*. 1999b
German	TT = ↑ FFA	glucose, insulin, FFA, ISI. OGTT: 2h glucose, insulin.	ni	ni	
Danes glucose tolerant	DM2 = ↑ % T carriers vs. glucose-tolerant subjects	glucose, insulin, OGTT: 30min & 2h & AUC for glucose & insulin, HOMA	TT = ↓ SBP, DBP	TC, TG, HDL, LDL, HT	Andersen *et al*. 2006
Italian HT pt	-	glucose	-	TC, TG, HDL, BP	Sartori *et al*. 2003
Americans	ni	ni	-	HT	Grove *et al*. 2007
Hispanic American					
Mixed Brazilians[‡]	-	DM %	Obese T carriers = ↑ SBP vs. obese CC	BP, HT, TC, LDL, HDL, VLDL, TG	Danoviz *et al*. 2006
Mexicans	TT = ↓ glucose, ↑ insulin sensitivity	-	-	BP, TC, TG HDL, LDL	Kopf *et al*. 2008

Table 2.35: *continued*

Population	Glucose and insulin homeostasis		Blood lipids and blood pressure (BP)		Reference
	Associations	No associations	Associations	No associations	
Native American					
Canadian Nunavut Inuit	ni	ni	-	BP, HT	Hegele *et al*. 1999
Canadian Oji-Cree	ni	ni	TT = ↓ SBP		Hegele *et al*. 1998
African					
African-American	ni	ni	-	HT	Grove *et al*. 2007
Asian					
Japanese	-	glucose, DM2	TT = ↑ SBP TT = ↓ % with no disorders (including obesity, HT, DM2, hypertriglyceridemia)	TC, TG, HT,	Yamamoto *et al*. 2004
Japanese DM2 pt	-	glucose, HbA1c, duration of DM, DM complications	-	HT	Hayakawa *et al*. 2007
Japanese	-	glucose, HOMA-IR, FFA	TT = ↓ DBP	TC, TG, HDL, SBP	Hayakawa *et al*. 2007
Chinese HT or cases	-	glucose	-	TC, TG, LDL, HDL, BP	Huang *et al*. 2003
Korean BMI>25	ni	ni	TT = ↑ SBP, ↓ VO_2max	DBP	Lee *et al*. 2005b
Korean BMI≤25	ni	ni	-	BP, VO_2max	Lee *et al*. 2005b
Chinese EH & NT	-	glucose	-	TG, TC, BP, HT	Wang *et al*. 2005

[‡] Consisting of Caucasian, African and Mulatto ancestry, DM2 = Type 2 Diabetes Mellitus, TG = triglycerides, TC = Total Cholesterol, HOMA-IR = Homeostasis model assessment of insulin resistance, HDL = High density lipoprotein, VLDL = Very low-density lipoprotein, LDL = Low density lipoprotein, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, FFA = free fatty acids, CAD = Coronary artery disease, OGTT = oral glucose tolerance test, AUC = area under curve, ISI = insulin sensitivity index, MI = myocardial infarction.

2.8.8 Associations with weight loss outcomes

Two weight loss interventions investigating the association between the *GNB3* C825T polymorphism and weight loss outcome were traced (Table 2.36). Both studies were performed on Caucasian populations using different weight loss interventions, including bariatric surgery (Potoczna *et al*. 2004) and Sibutramine treatment (Hauner *et al*. 2003). Both studies involved long-term follow-up of these patients. The patients undergoing bariatric surgery were prospectively followed for three years following the surgery and received the same initial and follow-up weight management information from a multidisciplinary health team. After three years, all patients, irrespective of the *GNB* C825T polymorphism, experienced the same weight loss and therefore this study concluded that this variant does not influence weight loss outcome (Potoczna *et al*. 2004). Although it was also mentioned that patients who experienced insufficient weight loss after the bariatric surgery were in addition treated with Sibutramine, Orlistat or a re-operation to improve weight loss, the number of patients receiving these additional treatment interventions did not differ between the genotype groups (Potoczna *et al*. 2004). In contrast, Hauner *et al*. (2003) reported an association between the *GNB3* C825T polymorphism and weight loss outcome in patients who were randomly assigned to a Sibutramine or placebo treatment intervention for 54 weeks. Both groups received individualized counselling sessions from a dietitian, a weight loss diet, 16 physical activity group sessions and four educational sessions on healthy eating, behaviour modification and motivation (Hauner *et al*. 2003). Long-term follow-up measurements were taken two years after the termination of the 54-week intervention. It was found that patients with the TT or TC genotype benefited from the conservative weight loss intervention without pharmacological intervention, while CC homozygous patients only benefited from Sibutramine therapy to lose weight (Hauner *et al*. 2003).

Several studies investigated the associations between the *GNB3* C825T polymorphism and the effects of exercise on obesity development. Grove *et al*. (2007) pointed out that TT homozygous African-Americans may especially benefit from physical activity to decrease their risk for obesity development. Using the Baecke physical activity questionnaire, these authors found that in African-Americans with low physical activity levels, each 825T allele contributes to a 23% higher obesity prevalence and therefore the TT homozygous subjects had the highest risk for obesity compared to the CC and CT subjects. However, in the high physical activity group the TT homozygous subjects had the lowest risk for obesity with each 825T allele being associated with a 20% lower obesity prevalence. In contrast, the CC subjects with high or low physical activity levels had the same relative risk for obesity development (Grove *et al*. 2007). In this research it was also shown that TT homozygous African-Americans may also benefit from being active and lean to decrease their risk for hypertension. The TT homozygotes who were either obese or inactive or both had a higher relative risk for hypertension, while TT homozygotes who were active and non-obese had a decreased hypertension risk (Grove *et al*. 2007). Rankinen *et al*. (2002) found that a 20-week endurance exercise programme was associated with greater decreases in fat mass and percentage body fat in TT homozygous African-Americans compared to C-allele carriers. Poston *et al*. (2002) reported that TT homozygous subjects with African ancestry were more obese than C-allele carriers regardless of their physical activity levels. Inconclusive results were also reported for Caucasian populations

with no associations between physical activity, obesity, body fat content or hypertension found in large samples including 10988 (Grove *et al*. 2007) and 473 (Rankinen *et al*. 2002) Caucasian-Americans. In contrast, Gutersohn *et al*. (2000) reported that high physical activity levels counteracted the susceptibility to a higher BMI after pregnancy in Caucasian TT homozygous women. However, when TT homozygous women were physically inactive, they suffered from a significantly higher post-pregnancy BMI as well as weight retention (Gutersohn *et al*. 2000). These findings are in line with those reported by Hauner *et al*. (2003) that TT homozygous Caucasian subjects benefit from a conservative weight loss intervention (that includes physical activity) to lose weight. In populations with Asian ancestry, no association was found between the *GNB3* C825T polymorphism, exercise duration, obesity and hypertension prevalence in Korean obese and non-obese men. However, it was shown that obese TT homozygous men and non-obese 825T carriers had a lower VO_2 max than the other genotype groups (Lee *et al*. 2005b).

In summary, in Caucasians one study found that the *GNB3* C825T polymorphism had no effect on weight loss outcome. The other study suggests that patients with the TT or TC genotype lose weight with a conservative weight loss intervention while CC homozygotes need in addition to this also Sibutramine to lose weight. Exercise interventions in Caucasian and African Americans also point in the direction that TT homozygous individuals may benefit from exercise to prevent obesity development or to decrease fat mass and percentage body fat. More research is needed to confirm these results and to investigate these associations in other populations.

Table 2.36: The association between the *GNB3* C825T polymorphism and change in weight (BMI) and other variables following a weight loss intervention.

Population	Sample size	Genotype frequencies	Inclusion criteria	Weight loss intervention	Variables measured	Results	Reference
Caucasian: Switzerland	N=304 ♀= 246 ♂= 58	TT=13% CC=47% TC=40%	BMI≥35	❑ Gastric banding surgery pt followed prospectively for 3 years. ❑ Pt was assessed by physician, bariatric surgeon, dietician, psychologist before operation. ❑ Office visits & weighing (no indication how often) ❑ No further description. ❑ Bariatric surgery: gastric banding and pt with insufficient weight loss (defined in article) was also treated with Sibutramine (n=37; 12.2% of pt) for 8.3±1.0 months or Orlistat (n=72; 23.7% of pt) for 14.9±1.9 months or reoperation (n=74).	***Measured at 0 & 36 months:*** Weight loss (kg), change in BP, change in HT %, nr of reoperations, nr treated with sibrutamine, nr treated with orlistat	No significant association between the CC, TC, and TT genotype groups for these variables. Weight loss = 32.4 kg (CC), 30.8 kg (CT), 31.2 kg (TT)	Potoczna *et al*. 2004
Caucasian: Germany	Sibutramine n = 52 Placebo: n=59	TT=14% CC=43% TC=43%	BMI≥30	***54 week intervention*** ❑ Pt randomly assigned to receive Sibutramine or placebo for 54 weeks. ❑ All pt: individualized counselling by dietitian ❑ Diet = 500-1000 kCal less than estimated requirements ❑ First 4 weeks: 4 educational sessions on healthy eating, PA, behaviour modification, motivation. ❑ PA = 16 groups sessions ❑ Follow-up 2 years after termination of intervention.	***Measured at 0, 54 weeks & 2 years thereafter:*** Baseline weight, BMI. After 54 weeks: weight loss, ≥5% and <5% weight loss, ≥10% and <10% weight loss. 2 year follow-up: ≥5% and <5% weight loss.	***Weight loss over 54 weeks intervention*** ❑ Sibutramine group lost 5.0 kg more than placebo ❑ Weight loss in placebo group: TT or TC lost 4.3 kg more than CC ❑ TT or TC do not benefit from Sibutramine ❑ CC + Sibutramine = 7.2 kg loss vs. 4.1 kg in T carriers. ***2 years follow-up after intervention termination*** ❑ Sibutramine no benefit over placebo ❑ Placebo: TT or TC = ↑ OR of ≥ 5% weight loss after 2y ❑ Sibutramine: CC = ↑ OR of ≥ 5% weight loss after 2y, while no benefit for TT or TC	Hauner *et al*. 2003

A54A = Ala54 homozygotes, A54T = heterozygotes, T54T = Thr54 homozygotes, T54 = Thr54 allele carriers, BMI = body mass index, WC = waist circumference, HC = hip circumference, WHR = waist-hip ratio, BW = body water, FM = fat mass, FFM = fat free mass, BP = blood pressure, SBP = systolic blood pressure, TC = total cholesterol, HDL = high density lipoprotein, LDL = low density lipoprotein, TG = triglycerides, RMR = resting metabolic rate, BG = blood glucose, VWAT = visceral white adipose tissue, SWAT = subcutaneous white adipose tissue.

Chapter 3

Methodology and

Experimental procedures

3.1 Study design

A cross-sectional study design was used to investigate the association between genotype and BMI, health and lifestyle indicators in overweight/obese Caucasian adults. For these purposes a cross-sectional sample of overweight/obese subjects who showed interest in participating in a weight loss programme was recruited and assessed. To investigate the association between genotype and weight loss outcomes after exposure to the treatment, a quasi experimental study design (time-series) was used. Subjects from the cross-sectional sample who volunteered to participate in the weight loss programme were exposed to the treatment (Figure 3.1).

The treatment consisted of a conservative weight loss programme that spanned 24 weeks, 16 weeks of active intervention (biweekly group sessions) followed by eight weeks of self-management (Figure 3.1). The subjects were expected to follow the weight loss recommendations during both phases until their goal weight has been reached. The assessments conducted on the cross-sectional sample (Table 3.1) also served as baseline assessments for the intervention sample. The cross-sectional data were collected prior to the commencement of the weight loss programme and during the first group session, which also served to explain the intervention to the volunteers. The cross-sectional sample included 133 subjects of whom 88 volunteered to participate in the intervention leg of the study. Follow-up data for the intervention sample were collected at the 16-week group session and the final weight measurement was taken at the 24-week group session.

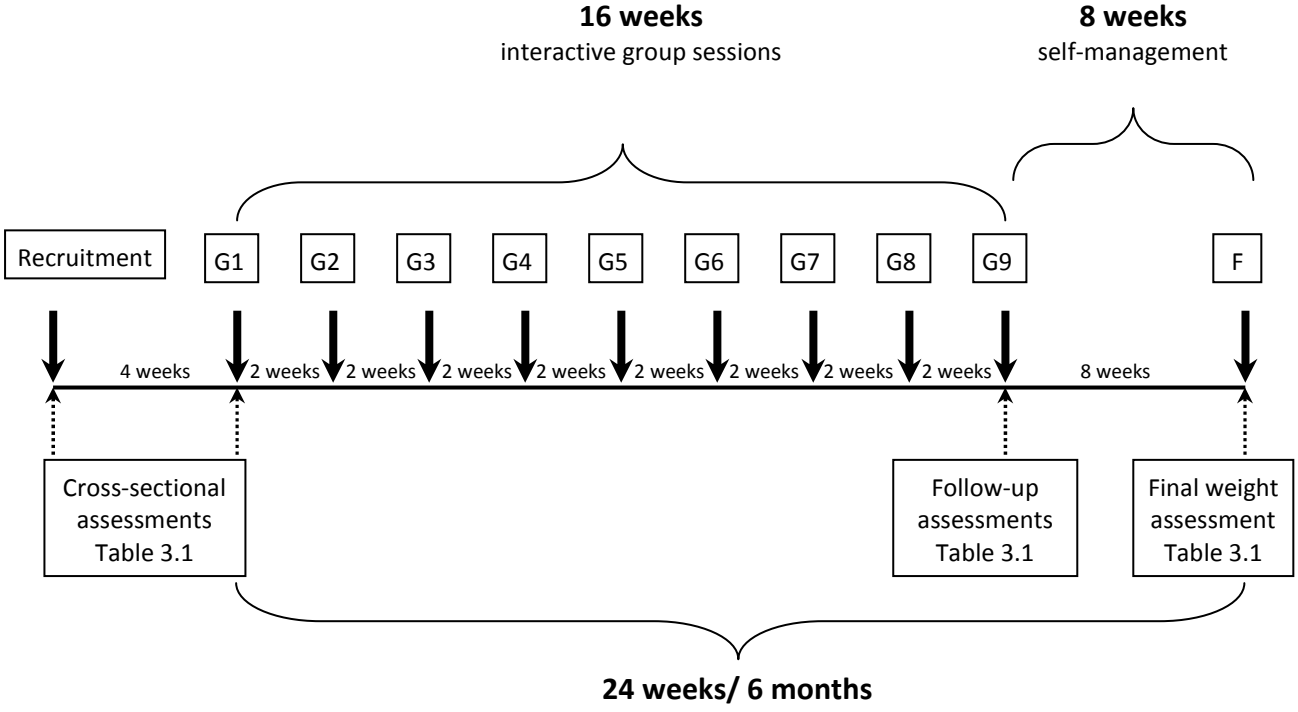

Figure 3.1: Time-line of the conservative weight loss intervention programme that spanned 24 weeks. Abbreviations: G = group session therefore G1 = group session 1; F = final group session.

A summary of the cross-sectional and follow-up assessments is presented in Table 3.1

Table 3.1: Summary of the cross-sectional and follow-up assessments.

Measuring tool/ technique	Cross-sectional variables 0 weeks	Follow-up variables 16 weeks	 24 weeks
Blood samples: DNA extraction and genotype analyses	Genotype of 7 polymorphisms: *FABP2* Ala54Thr, *INSIG2* rs7566605, *FTO* rs17817449, *FTO* rs1421085, *ADRB3* Trp64Arg, *ADRB2* Arg16Gly, *GNB3* C825T.		
Anthropometric measurements	Weight and height BMI	Weight BMI	Weight BMI
Questionnaire developed for this research	*Socio-demographic:* Age, gender, marital status, home language, highest education levels, living alone or with friends/family.	-	-
	Perceived weight history: Perception of weight during childhood, adolescence and young adulthood. Perception of parents' current weight and weight history.	-	-
Non-quantified food frequency questionnaire	Dietary intake of indicator food groups prior to intervention	-	-
Baecke questionnaire	Physical activity: Work index, sport index, leisure-time index	Physical activity: Work index, sport index, leisure-time index	-
3-Factor Eating Questionnaire	Eating behaviour: Dietary restraint, disinhibition and perceived hunger	Eating behaviour: Dietary restraint, disinhibition and perceived hunger	-
Beck Depression Inventory	Depression	Depression	-
Rosenberg Self-Esteem Scale	Self-esteem	Self-esteem	-
General Health questionnaire	General psychological well-being	General psychological well-being	-
Anthropometric measurements	Waist circumference	-	-
Blood pressure	Blood pressure	-	-
Blood samples	Fasting HDL-cholesterol, triglycerides and glucose (for metabolic syndrome diagnosis).	-	-

3.2 Study population

The study population consisted of Caucasian South African overweight and obese subjects residing in Cape Town and surrounding areas. In this study "Caucasian" South Africans refers to individuals of European descent, mainly Dutch, French, German and British origin.

3.2.1 Inclusion and exclusion criteria

A brief screening questionnaire (Addendum A) was used to screen potential subjects for inclusion in the study.

Subjects had to have a BMI ≥27 kg/m^2 and be between the ages of 25 and 40 years old to be included in the study. Subjects older than 40 years were excluded because weight gain patterns in this age group might be different compared to younger people (Korkeila *et al*. 1995).

The exclusion criteria included the following: pregnancy or currently breastfeeding, a history of major eating disorders, serious psychiatric illness, a history of drug or alcohol abuse, treatment with any drugs for weight loss in the preceding four weeks, presence of comorbidities of obesity such as diabetes and Cushing's syndrome and inability to exercise.

3.2.2 Subject recruitment

Subjects were recruited by means of advertisements placed in local newspapers, the e-mail bulletins of the Universities of Stellenbosch and Cape Town and by word of mouth. Targeted newspapers included "Die Burger" (an Afrikaans newspaper circulating in Cape Town and other towns in the Western Cape), "Cape Times" (an English newspaper circulating in Cape Town and other towns in the Western Cape), "District Post" (a regional newspaper circulating in the Helderberg region including Somerset-West, Strand, Gordons Bay and Stellenbosch) and "Eikestadnuus" (a regional newspaper circulated mainly in Stellenbosch, but also available in the Helderberg region).

The newspaper advertisements indicated that individuals should phone or e-mail the primary researcher for more information on the study. On contact the study was briefly explained to the individual and the screening questionnaire was administered either telephonically or via e-mail. This procedure served to exclude individuals who clearly did not meet the inclusion/exclusion criterions e.g. if a person did not meet the age requirement. Appointments for individual recruitment interviews were made with all potential subjects who seemed to meet the inclusion criteria. At the recruitment interview potential subjects received detailed information regarding the project, the consent form (Addendum B) was explained to them and they had the opportunity to ask questions for clarification. After subjects signed the consent form, their weight and height were taken. If their

BMI was ≥27 kg/m^2 and the other inclusion criteria were met, the subject was included in the cross-sectional sample. The necessary assessments (Table 3.1) were completed during the recruitment interview and at group session one. An appointment was also made for the collection of a fasting blood sample. The recruitment period stretched over a period of approximately one month before a particular intervention leg started (see section 3.3.3 for explanation). Subjects were phoned to confirm the date, time and place of the first group session. Over and above the completion of some of relevant assessments during the first group session, a detailed explanation of the actual intervention was provided to subjects, after which they could finally choose to continue with the weight loss intervention or to withdraw from the study. The final sample of overweight and obese individuals who responded to the advertisements and who completed all cross-sectional assessments is 133. Of these, 88 chose to continue with the weight loss intervention.

3.3 Treatment

3.3.1 Programme content

All subjects received the same conservative weight-loss treatment that included a balanced weight-loss diet, physical activity recommendations as well as behavioural and cognitive strategies to facilitate behaviour change. These topics as well as the effective application thereof were discussed in biweekly group sessions that stretched over a period of four months (nine group sessions in total) (see Figure 3.1 and Table 3.2). At the first group session the subjects received general weight loss guidelines regarding dietary intake, physical activity and behavioural strategies that they needed to follow for the 24-weeks intervention period. Each subject received the self-help weight management manual by Senekal (2005) and they were advised to work through the manual in their own time before the next session to identify their personal weight management needs, goals and relevant actions as is indicated in the manual.

3.3.2 Individualisation of energy and dietary recommendations

The energy requirement for weight loss for each subject was calculated by subjects themselves using the equation recommended by Senekal (2005) that considers current weight, physical activity level and gender. Based on the outcome of this calculation and following review by a registered dietitian, the participant was advised to follow a 4200, 5000, 5800, 6600 or 7400 kilojoules diet with a macronutrient distribution of 50% carbohydrates, 20% protein and 30% fat. The meal plans for each diet as described in the manual by Senekal (2005) were used. The meal plan for each energy level specifies the number of daily servings from the six food groups (starch, protein, fruit, vegetables, milk and fat/extras). An example of a menu plan for one day for each energy level as well as portion sizes for a single serving of common foods and a graphic guide for portion size estimation are provided in the manual (Senekal 2005). During the first group session subjects were guided by the dietitian to select an appropriate eating plan to meet their calculated energy intake goal, plan a menu and

use the portion size estimation guidelines. Subjects were also provided with menu examples for one week, after which they were expected to compile their own menus using a template that covered a week.

Table 3.2: Topics and activities covered during group sessions in the weight loss programme.

Session	Topics	Main activities
1	Diet Healthy eating Reasonable weight goal	**Subjects:** ❑ Complete baseline questionnaires. ❑ Receive weight management manual (WMM): Love my body, love myself (Senekal 2005) ❑ Calculate energy goals (each subject) ❑ Complete questions in WMM to determine a reasonable weight goal. **Group leader:** ❑ Explains diet, food groups, portion sizes, meal plans, menu planning and basic healthy eating tips. ❑ Provide general guidelines for dietary intake, physical activity and behavioural strategies necessary for weight loss.
2	Glycemic index of foods Fibre content of foods Eating behaviour & healthy eating habits	**Group leader:** ❑ Explains the effect of different food on blood glucose. ❑ Discusses hand-out with GI food lists. ❑ Discusses fibre recommendation and amount and type of food that needs to be consumed to reach this goal. ❑ Compares fibre content of different breakfast cereals and porridges, fruit, vegetables and starches. ❑ Facilitates discussion on unhealthy eating behaviours **Subjects:** ❑ Identify unhealthy eating behaviours from checklist in WMM
3	Physical activity	❑ **Exercise educationalist** demonstrates activities/ exercises and motivates group to increase their activity levels.
4	Visualisation, self-concept, body image, assertiveness, stress management, communications skills, writing goals, self-motivation manage environmental cues	**Subjects:** ❑ Work through exercises covering concepts related to behavioural and psychological components influencing weight loss. **Group leader:** Facilitates discussion of these concepts and problem areas identified by subjects.
5	Reading labels Take-away foods	**Group leader:** ❑ Compares the energy and macronutrient content of different take-away foods (e.g. McDonalds, Steers, Nando's, King Pie etc.) with macronutrient composition of their food groups.
6	Weight loss methods	**Group leader:** ❑ Facilitates discussion on over-the-counter drugs for weight loss and "famous" weight loss diets.
7	Cooking demonstration Recipe adaptations	**Group leader:** ❑ Executes a cooking demonstration ❑ Facilitates discussion on how to include more legumes and soy in meals. ❑ Facilitates discussion on how to make recipes healthier. **Subjects:** ❑ Work through two recipes and suggest healthy adaptations.
8	kJ content of drinks	**Group leader:** ❑ Compares the energy content of different alcoholic beverages, soft drinks, juices, yoghurts, and milk drinks
9	Quick dinners from frozen meals	**Group leader:** ❑ Compares the nutritional content of several frozen meals. **Subjects:** ❑ Complete follow-up questionnaires.

3.3.3 Procedures and physical arrangements

During the years 2006 and 2007 group sessions were conducted at four different venues starting at 15 different time-points. All group sessions were facilitated by registered dietitians (three in total). The venues for group sessions were located in Stellenbosch (three weight loss groups during 2006 and five weight loss groups during 2007), Strand (two weight loss groups), Cape Town (two weight loss groups), and Tygerberg (three weight loss groups). A dietitian was allocated to a particular group for the duration of the intervention to ensure continuity and the development of trust and a treatment relationship between the dietitian and subjects. Dietitians were responsible for phoning subjects before each group session to remind and motivate them to attend the upcoming session. Subjects were allowed to contact respective dietitians at any stage during the 24 week intervention via telephone or e-mail regarding any weight management related queries. Trained fieldworkers assisted with the collection of baseline and follow-up assessments, the running of the group sessions as well as monitoring of completion and hand in of perceived compliance forms.

3.4 Selection of genes

The genes investigated in this study, which forms part of a larger investigation of in both Caucasian and Zulu subjects, were selected based on 1) the criteria for selecting genes and polymorphisms for this type of research proposed by Day (2004) and 2) personal communications with experts in the field namely Drs. L Perusse (Division of Kinesiology, Department of Social and Preventive Medicine, Faculty of Medicine, Laval University, Quebec, Canada), A Peeters (Center for Medical Genetics, Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium) and MJ Kotze (Department of Pathology, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa). Application of the criteria formulated by Day (2004) as well as input from the panel of experts in the selection of the candidate genes for this study can be summarized as follows:

Criterion 1: The gene product plays an important role in physiological pathways that might contribute to weight gain and obesity development (Day 2004).

- Gene products involved in fat absorption (*FABP2*), the regulation of fat storage and fat oxidation (lipid turnover) in adipose tissue (*ADRB3*, *ADRB2* and *GNB3*) and adipogenesis (*INSIG2*) were deemed relevant for inclusion in this research.

Criterion 2: Evidence from *in vivo* experiments has shown that under- (knockout) or over- (transgenic) expression of the gene in animals influences obesity development (Day 2004)

- The required evidence is available for all the above selected genes (see physiological function sections of each of the relevant genes in Chapter 2).

Criterion 3: Evidence exists for a gene-diet interaction, either from cross-sectional studies or, more importantly, from intervention studies (Expert panel).

- A list of genes associated with weight loss as a result of a weight loss intervention was compiled from available literature. From this list it was evident that the associations between weight loss outcome and polymorphisms in the *ADRB3*, *GNB3* and *FABP2* genes have been previously investigated.
- Relevant evidence in this regard is presented in Chapter 2 for each of these genes.

Criterion 4: Priority must be given to genes with the most consistent evidence of an association with obesity (Expert panel).

- A list of genes that showed positive associations with an obesity related phenotype indicator by more than **five** studies was compiled from the 2006 Obesity gene map (Rankinen *et al*. 2006) (Table 2.3 in Chapter 2). From this list it was evident that the associations between *ADRB3*, *ADRB2* and *GNB3* and obesity phenotype indicators were consistent.
- It was recommended by the expert panel that the *FABP2* gene should also be included in the study as a novel gene. At that point in time (2007) the association between obesity phenotype indicators and the *FABP2* gene was supported by four studies. However, further supportive evidence has since been published (Section 2.3.6 in Chapter 2).

Criterion 5: Genome-wide linkage studies have mapped the gene to a chromosomal region associated with obesity development (Day 2004).

- This is true for *ADRB3*, *ADRB2*, *GNB3* and *FABP2*, as is evident from the 2006 Obesity gene map (Rankinen *et al*. 2006).

Criterion 6: Promising novel genes identified through Genome-wide association studies should be investigated in lieu of the promotion of basic research (Expert panel)

- The expert panel recommended the inclusion of *FTO* and *INSIG2* genes.

When selecting candidate genes for a particular research study it must be borne in mind that more than one polymorphism in a particular gene may be associated with obesity phenotype indicators (Day 2004). For the purposes of this research priority was given to functional polymorphisms with a higher reported frequency in Caucasian and/or African (i.e. Zulu) populations that showed the strongest evidence for an association with an obesity related phenotype. Novel polymorphisms of the *FTO* and *INSIG2* genes were also selected although their functionality is still under investigation.

3.5 Collection of whole blood samples

Blood was drawn from each subject following a ten hour overnight fast by trained phlebotomists. Blood samples were collected in three BD (Becton, Dickinson and Company) vacutainers, namely an ethylene diamine tetra-

acetic acid (EDTA) tube for DNA analysis, a tube containing succinylsulfathiazole (SST) and gel for testing HDL cholesterol and triglyceride levels and a tube containing sodium fluoride for testing blood glucose levels.
Blood analyses for glucose, HDL-cholesterol and triglyceride levels were conducted by a pathology laboratory, Dietrich, Street, Loftus & Partners (Pathcare laboratories) on the day the samples were collected.

3.6 Genotype analysis[3]

3.6.1 DNA extraction from whole blood

The protocol of Miller *et al*. (1988) was modified to extract DNA from the whole blood samples collected in this study.

Each 10 ml whole blood sample was transferred from the EDTA tube into a 50 ml polypropylene Falcon tube (Greiner Bio-One, Cellstar®) and 30 ml ice cold lysis buffer [155 mM ammonium chloride (NH_4Cl), 10 mM potassium hydrogen carbonate ($KHCO_3$), 0.1 mM EDTA, pH 7.4] was added. Each Falcon tube with the solution was inverted and then placed on ice for 30 minutes, with inversion every 10 minutes to ensure complete lysis of the red blood cells. The tubes were then centrifuged at 1750 $\times$ *g* for 10 minutes. After discarding the supernatant, the pellet was washed with 10 ml phosphate buffered saline (PBS) solution and centrifuged at 1750 $\times$ *g* (1500 rpm) for 10 minutes. The supernatant was discarded and the pellet dissolved by adding 3 ml nuclear lysis buffer [10 mM Tris hydrochloride (Tris-HCl), 400 mM sodium chloride (NaCl), 2 mM EDTA, pH8.2], 10mg/ml Proteinase K (Roche Diagnostics) and 1% (w/v) sodium dodecyl sulphate (SDS). The solution was incubated at 55^{o}C in a water bath overnight.

Following incubation, 1ml saturated 6 M NaCl was added to each Falcon tube. The tubes were shaken vigorously for one minute and then centrifuged at 3250 $\times$ *g* for 30 minutes. The supernatant was transferred into a new Falcon tube and the remaining foam and pellet were discarded. The Falcon tube was shaken for 15 seconds and then centrifuged at 2250 $\times$ *g* for 15 minutes. The supernatant was transferred into another new Falcon tube and the remaining foam and pellet were discarded. Two volumes ice-cold ±99.9% (v/v) ethanol (EtOH) were added to the supernatant to precipitate the DNA. The spool of DNA formed was scooped out of the tube and placed into a new 1.5 ml Eppendorf tube with 70% (v/v) EtOH. This was centrifuged at 20800 $\times$ *g* for 10 minutes at 4 ^{o}C for removal of excess salt. After discarding the ethanol, the pellet was allowed to air-dry before dissolving it in 250 µl SABAX water. To completely dissolve the DNA the suspension was shaken overnight at room temperature. The DNA concentration of each suspension was measured with a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies). Subsequently, the DNA was diluted with SABAX water to a concentration of 50 ng/µl and stored at 4 ^{o}C.

[3] The candidate performed all the DNA extractions and the genotyping of the FABP2 Ala54Thr and ADRB3 Trp64Arg polymorphisms

3.6.2 Polymerase chain reaction (PCR) amplification

A summary of the oligonucleotide primers used for PCR amplification is shown in Table 3.3. All primer sets were manufactured by Integrated DNA Technologies (IDT) and the melting temperature (T_M) for each primer was calculated using the equation [$T_M = 2(nA + nT) + 4(nG + nC)$] described by Thein and Wallace (1986).

PCR amplification was performed using a GeneAmp® 2700 PCR System (Applied Biosystems). Each PCR reaction was performed in a volume of 25 µl consisting of 50 ng DNA, 0.2 mM of each dNTP (dATP, dCTP, dGTP, dTTP) (Fermentas), 10 pmol of each primer, magnesium chloride ($MgCl_2$) and *Taq* polymerase (as indicated in Table 3.3) and 1×*Taq* buffer (Fermentas or Bioline). The various amplicons under investigation were amplified using one of the following PCR cycles (referred to as cycle A or B):

- Cycle A consisted of an initial denaturation at 95°C for five minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55 °C for 45 seconds and elongation at 72°C for 30 seconds. The final extension step occurred at 72°C for ten minutes.
- Cycle B consisted of an initial denaturation at 95°C for two minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and elongation at 72°C for 30 seconds. The final extension step occurred at 72°C for ten minutes.

3.6.3 Agarose gel electrophoresis

To investigate whether PCR amplification was successful, 5µl of each PCR product was mixed with 5µl cresol red loading buffer (2 mg/ml cresol red and 35% (w/v) sucrose). The solution was loaded onto a 1% (w/v) horizontal agarose gel [2g agarose in 200 ml 1×TBE (90mM Tris-HCl, 90 mM boric acid (H_3BO_3) and 0.1 mM EDTA, pH 8.0) and 0.01% ethidium bromide (EtBr)]. A 100 bp ladder (Fermentas) was also loaded onto the gel to confirm PCR amplification of the correct fragment size. Electrophoresis was performed at 120 V for one hour in 1×TBE buffer solution and then visualised by ultraviolet light transillumination (GeneSnap MultiGenius Bio Imaging System, Syngene).

3.6.4 Restriction fragment length polymorphism (RFLP) analysis

To identify the specified genotypes for the genes under investigation, RFLP analysis was performed. Digestion of the respective PCR products was performed in a 20 µl reaction containing 10 µl PCR product, units of the relevant enzyme as shown in Table 3.4 and 1×buffer. This was placed in a water bath at the temperature and time as shown in Table 3.4. The digested PCR products were loaded onto an agarose gel (gel concentrations shown in Table 3.4) and visualized by ultraviolet light transillumination (GeneSnap MultiGenius Bio Imaging System, Syngene). Digestion results for all three genotypes (wild-type, heterozygous, mutant) of each of the seven polymorphisms were confirmed by Inqaba Biotechnical Industries (Pty) Ltd using sequencing analysis.

Table 3.3: Specific oligonucleotide primers used and PCR conditions implemented.

Genes: Polymorphism	Primer sequences (5′ – 3′)	Reference(s)	T_M (^oC)	Amplicon size (bp)	PCR cycle	$MgCl_2$ (mM)	*Taq* polymerase	Success[§] n(%)
FABP2: Ala54Thr	(F) ACAGGTGTTAATATAGTGAAAAG (R) TACCCTGAGTTCAGTTCCGTC	Baier *et al*. 1995 Kim *et al*. 2001	48 56	180	A	1	Bioline	112(84.2)
INSIG2: rs7566605	(F) CCTACCTCCCTCCAATACCC (R) TGAGAGTCAGTGCGATGTCC	Feng *et al*. 2007	56 57	164	A	2.5	Fermentas	115(86.5)
FTO: rs1421085	(F) TAGTAGCAGTTCAGGTCCTAAGGCGTG* (R) CAGATTAAGGTGATGGGTTG	This study[†]	61 51	240	A	2	Fermentas	101(75.9)
FTO: rs17817449	(F) AGGACCTCCTATTTGGGACA (R) AGCTTCCATGGCTAGCATTA	Do *et al*. 2008	55 54	828	A	2	Fermentas	105(79.0)
ADRB3: Trp64Arg	(F) CAATACCGCCAACACCAGTGGG (R) GGTCATGGTCTGGAGTCTCG	Gagnon *et al*. 1996 Hao *et al*. 2004	61 57	151	B	2	Fermentas	93(69.9)
ADRB2: Arg16Gly	(F) CTTCTTGCTGGCACGCAAT* (R) CCAGTGAAGTGATGAAGTAGTTGG	Large *et al*. 1997 Ukkola *et al*. 2000	57 55	200	A	1.5	Fermentas	106(79.7)
GNB3: C825T	(F) TGACCCACTTGCCACCCGTGC (R) GCAGCAGCCAGGGCTGGC	Siffert *et al*. 1998 Buchmayer *et al*. 2000	65 66	268	A	2	Fermentas	107(80.5)

PCR = polymerase chain reaction, (F) = forward primer, (R) = reverse primer, T_M = melting temperature, bp = base pairs, $MgCl_2$ = magnesium chloride.

*Mutagenic primers; [†] The mutagenic primer was designed using *In Silico*.

[§]Indicates the number of samples for which genotyping was successful

Table 3.4: Specific restriction enzymes and conditions used for RFLP analysis

Gene: polymorphism (amino acid change)	Units and enzyme (Manufacturer)	Recognition site	Fragments	Water bath: Incubation time & temperature	Agarose gel: Concentration, voltage & electrophoresis time
FABP2: Ala54Thr	2U *Hha*I (NEB)	5'-GCG↓C-3'	GG (Ala/Ala) = 99, 81bp AA (Thr/Thr) = 180bp GA (Ala/Thr) = 180, 99, 81bp	2 hours at 37^{o}C	4% gel 80V for 2 hours
INSIG2: rs7566605	5U *Dpn*II (NEB)	5'-↓GATC-3'	CC = 88, 76bp CG = 164, 88, 76bp GG = 164bp	Overnight at 37^{o}C	3% 120V for 1 hour
FTO: rs1421085	1U *Mae*III (Roche)	5′-↓GTNAC-3′	TT = 240bp CC = 216, 24bp TC = 220bp, 216bp, 24bp	Overnight at 55^{o}C	3% 120V for 1 hour
FTO: rs17817449	2U *AlwN*I (Fermentas)	5'-CAGNNN↓CTG-3'	GG = 240bp TT = 216, 24bp GT = 220, 216, 24bp	Overnight at 37^{o}C	1.5% 120V for 50 min
ADRB3: Trp64Arg	2U *Msp*I (NEB)	5′-C↓CGG-3'	TT (Trp/Trp) = 97, 54bp CC (Arg/Arg) = 70, 54, 27bp TC (Trp/Arg) = 97, 70, 54, 27bp	Overnight at 37^{o}C	3% gel 120V for 1 hour
ADRB2: Arg16Gly	2U *BsrDI* (NEB)	5'-GCAATGNN↓-3'	AA (Arg/Arg) = 131, 56, 14bp AG (Arg/Gly) = 131, 108, 56, 22, 14bp GG (Gly/Gly) = 108, 56, 22, 14bp	Overnight at 65^{o}C	3% 120V for 1 hour
GNB3: 825C→T	2U BseDI (Fermentas)	5'-C↓CNNGG-3'	TT = 268bp CC = 152, 116bp CT = 268, 152, 116bp	Overnight at 55oC	3% 120V for 1 hour

3.7 Anthropometric assessments

Weight, height and waist circumference of subjects were measured by trained and standardized fieldworkers. Weight was measured in light clothing without shoes to the nearest 0.1 kg using a calibrated electronic scale with a 250 kg capacity (Physician scale, Scales 2000). Height without shoes was measured to the nearest 0.1 cm with a stadiometer. The participants stood with their feet together and heels, buttocks, scapulae and back of the head touching the vertical surface of the stadiometer, if possible. Any hair obstructions were removed and the head was placed in the Frankfort horizontal plane. The body mass index (BMI) was computed as weight (kg)/ height $(m)^2$ (Bastow 1982). The BMI was categorized according to the World Health Organization guidelines, namely BMI $\geq$27 and <30 kg/m^2 = overweight; BMI$\geq$30 and <35 kg/m^2 = obese class I; BMI$\geq$35 and <40 kg/m^2 = obese class II and BMI$\geq$40 kg/m^2 = obese class III (WHO 2000).

Waist circumference was measured to the nearest 0.1 cm using a non-stretchable measuring tape. The waist circumference was taken at the level of the narrowest point between the lower rib border and the iliac crest after normal expiration. When there was no obvious narrowing point, the measurement was taken at the mid-point between the two landmarks (Norton *et al*. 1996). The subject stood relaxed with feet together, while the fieldworker ensured that the measuring tape was horizontal when the measurement was taken.

3.8 Diagnosis of the Metabolic syndrome (MetS)

At the time of data collection several definitions to diagnose MetS were available (reviewed by Stolar 2007). The most commonly used definitions included those set by the National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III), International Diabetes Foundation (IDF) and World Health Organization (WHO). Metabolic syndrome traits included in the definitions of the NCEP ATP III, IDF and WHO are waist circumference, blood pressure, fasting triglycerides, HDL-cholesterol and glucose. In addition to this the WHO include obesity, waist-hip-ratio, urinary albumin and albumin-creatinine ratio in their definition. Diagnostic cut-offs for each trait are specified by each organization, which vary for most traits, with the exception of cut-offs for triglyceride levels. For diagnosis of the metabolic syndrome the IDF criteria specify the presence of obesity and the WHO criteria specify the presence of insulin resistance together with two of the other traits. The NCEP ATP III criteria require any combination of three or more of the mentioned traits for diagnosis (ATP III 2001). For this reason it was decided to use the NCEP ATP III criteria (Table 3.5) to diagnose subjects with the metabolic syndrome in our research. This choice is also supported by the fact that the newly proposed harmonized criteria[4] cover the same traits as the NCEP ATP III criteria and also require any combination of three or more of these traits for diagnosis (Alberti *et al*. 2009).

[4] Joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity.

Table 3.5: NCEP ATP III criteria for the diagnosis of the metabolic syndrome (ATP III 2001).

Indicators of:	Measurement	Cut-offs for diagnosis
Central obesity	Waist circumference	Men: >102 cm; Women: >88 cm
Impaired glucose tolerance	Fasting glucose	≥6.1 mmol/L
Hypertriglyceridaemia	Fasting plasma triglycerides	≥1.69 mmol/L
	HDL cholesterol	Men: <1.04 mmol/L
		Women: <1.24 mmol/L
Hypertension	Blood pressure	≥130/85 mmHg or currently treated with hypertension medication

NCEP ATP III = Adult Treatment Panel third report of the National Cholesterol Education Programme

All measures for MetS diagnosis were only obtained from a sub-sample (n=88) of subjects. The n for MetS variables further vary due to no results received from Pathcare laboratories for HDL-cholesterol for four subjects as well as waist circumference and blood pressure measures not obtained from four subjects.

3.9 Physical activity

The Baecke Questionnaire of Habitual Physical Activity was developed by Baecke *et al*. (1982) to measure three components of physical activity, namely physical activity at work, sport during leisure time and physical activity during leisure time, excluding sport. The questionnaire was developed, calibrated and tested for reliability using men and women between the ages of 20 to 32 years from a white population in the Netherlands (Baecke *et al*. 1982). The test-retest reliability of the work, sport and leisure-time indices were 0.88, 0.81 and 0.74 respectively (Baecke *et al*. 1982). Other reliability studies showed similar or better test-retest results in male and female Caucasian university staff and students ages 20 to 59 years (Jacobs *et al*. 1993) and Dutch men and women aged 20 to 70 years (Pols *et al*. 1995). Validation studies also showed significant relationships between the Baecke physical activity indices and other physical activity questionnaires (Cauley *et al*. 1987, Albanes *et al*. 1990, Miller *et al*. 1994), energy intake (Albanes *et al*. 1990, Miller *et al*. 1994), VO_2max, % body fat, Caltrac readings and activity diaries (Richardson *et al*. 1995, Jacobs *et al*. 1993, Pols *et al*. 1995).

The Baecke Questionnaire is self-administered and consists of 16 questions. A score is computed for each of the three mentioned sections and referred to as the work index, sport index and leisure-time index. The score for the work index is calculated from the first eight items, for the sport index from items nine to 12 and for the leisure-time index from items 13 to 16 in the questionnaire. All items except items one and nine are answered by choosing one of five options, namely 'never', 'seldom', 'sometimes', 'often', or 'always/very often'. These responses are scored using a 5-point Likert scale. In item nine respondents are asked to report on the type of sport the subject practises most frequently as well as the number of hours per week and months per year the subject practises this sport (Baecke *et al*. 1982). Scoring of this item is based on the description supplied by Baecke *et al*. (1982). As the long-term pattern of habitual physical activity is measured by the questionnaire, the

mean scores of each index can be compared between follow-up measurements to reflect changes over time (Baecke *et al*. 1982).

3.10 Dietary intake

The aim of the dietary assessment was to characterize food choices at baseline to 1) direct dietary recommendations for the intervention and 2) to compare frequency of intake of energy-dense foods groups between genotype groups of subjects in the cross-sectional sample. For these purposes a **non-quantified** food frequency questionnaire (FFQ), including 67 items allocated to nine food groups, was developed (Addendum C). The nine groups included starches (nine items); vegetables (three items); fruit (four items); milk, yoghurt and cheese (five items); meat, fish, chicken (13 items); fats (four items); fast foods and take-away foods (five items); "other foods" (e.g. vetkoek, samoosas, koeksisters, doughnuts; muffin, scones, cake, tart; rusks; cookies; chips; chocolate; ice cream; sweets; energy bars, health bars and breakfast bars; nuts and peanuts; white, cheese and meat sauces; tomato sauce, chutney and other sauces; chocolate spread; peanut butter; jam, syrup and honey) (15 items); and drinks (alcoholic and non-alcoholic) (nine items). Food items included were selected to cover all the food groups, with emphasis on food items that have been shown to be associated with obesity development (Bray *et al*. 2004a, Rosmond *et al*. 2004, Black & Macinko 2008, Malik *et al*. 2006, Gibson 2008, Wolf & Dansinger 2008).

Frequency of intake of specific food items could be indicated as <1/month, 1-3 times/months, 1/week, 2-4/week, 5-6/week, 1/day, 2-3/day, 4-5/day, 6+/day. As it was a non-quantified FFQ, portion size estimation was not included. The daily frequency of intake of each item on the food list in the FFQ was calculated as shown in Table 3.6.

Table 3.6: Formulas for the calculation of daily frequency of food intake from the non-quantified FFQ

Intake		Calculation
Daily	❑ using daily intake as is	- if 1/day was selected daily intake = 1, - if 2-3/day = 2.5, - if 4-5/day = 4.5, - if 6+/day = 6.
Weekly	❑ dividing weekly intake by seven:	- if 5-6/week was selected daily intake = 5.5/7, - if 2-4/week = 3/7 - if 1/week = 1/7,
Monthly	❑ dividing monthly intake by 28:	- if 1-3/month was selected daily intake = 2/28
Never or <1/month	❑ use 0.	- 0

Energy-dense food items were grouped (Table 3.7) to calculate a daily intake of 1) high fat foods, 2) energy-dense snacks and 3) non-alcoholic drinks. Although take-away foods were included in the high fat food group,

these foods were also included as a separate fourth food group in statistical analyses. For data-analysis the daily mean±SD frequency of intake of each indicator food group was calculated.

Table 3.7: Allocation of food items to the three energy-dense food groups created for the purposes of this study.

New food groups	Original food groups on non-quantified FFQ	Single or groups of food items on non-quantified FFQ included in new food groups
High fat foods	❑ Starches	- Potato cooked, baked, mashed with fat added or potato salad.
	❑ Milk, cheese, yoghurt group	- Full cream milk, yogurt, sour milk; - Coffee creamer; - Cheese (gouda, cheddar, brie etc.)
	❑ Meat, fish, chicken	- Schnitzels, cordon bleu; - Red meat e.g. beef, mutton, pork (eat visible fat); - Chicken/turkey with skin; - Fried fish; - Sausages e.g. Vienna's, Russians, frankfurter; - Bacon and cold meat e.g. salami, polony; - Eggs e.g. scrambled, baked, omelettes.
	❑ Fats	- Soft margarine; - Butter or hard margarine; s - Salad dressing/ mayonnaise e.g. normal fat.
	❑ Take-away foods	- Pizza - Pies and sausage rolls; - Potato chips; - Kentucky; - Hamburgers e.g. McDonalds, Steers, Wimpy, Spur etc.
	❑ Other	- Cheese sauce, white sauce, meat sauce; - Chocolate spread;
Energy-dense snacks (= high fat and carbohydrate snacks)	❑ Other	- Vetkoek, samoosas, koeksister, doughnuts; - Muffin, scones, cake, tart; - Cookies; - Chips; - Energy bars, health bars, breakfast bars; - Chocolate; - Ice cream; - Sweets.
Energy-dense drinks (=non-alcoholic drinks)	❑ Drinks	- Juice - Fizzy soft drinks; - Energy drinks; - Milk shake.

3.11 Eating behaviour

The Three-Factor Eating Questionnaire (TFEQ) or Eating Inventory, was developed by Stunkard and Messick (1985) to measure three dimensions of human eating behaviour, namely dietary restraint (Factor I), disinhibition (Factor II) and perceived hunger (Factor III) (Stunkard & Messick 1985). The questionnaire can be used to study individuals and to detect group differences in eating behaviour. The questionnaire was developed and tested for

reliability using non-obese and obese men and women who were either very restrained or unrestrained eaters (Stunkard & Messick 1985). Internal reliability (Cronbach's α) of the TFEQ was 0.93 for Factor I, 0.91 for Factor II and 0.85 for Factor III (Stunkard & Messick 1985). The questionnaire has been validated in large cohorts consisting of non-obese and obese men and women (Laessle *et al*. 1989, Provencher *et al*. 2003).

The TFEQ is a self-report questionnaire that consists of 51 questions. Part I of the questionnaire consists of 36 true or false questions and Part II (question 37 to 51) consists of questions with four possible answers (with the exception of question 50 that has six possible answers). Respondents must choose one option for each question. Responses to all questions are scored as 0 or 1 and summed as explained by Stunkard and Messick (1985). Twenty one of the questions comprise Factor I (score ranging from 0 to 21) that measures dietary restraint (TFEQ-R) and reflects the extent to which food intake is cognitively restricted (by thought and will power) in order to control body shape and weight. Sixteen questions comprise Factor II (score ranging from 0 to 16) that measures disinhibition (TFEQ-D) and reflects the extent of inability to control food intake in response to the presence of palatable food which may result in the over-consumption of food. Other disinhibiting stimuli such as emotional stress or social eating cues may also contribute to the inability to resist food intake when not hungry. Fourteen questions comprise Factor III (score ranging from 0 to 14) that measures perceived hunger (TFEQ-H) and reflects the extent of food intake in response to susceptibility to general subjective feelings and perceptions of hunger and the behavioural consequences thereof (Stunkard & Messick 1985).

Specific subscales of these three general eating behaviours have been proposed (Westenhoefer *et al*. 1999, Bond *et al*. 2001). The TFEQ-R scale can be subdivided into five subscales, namely rigid control, flexible control, attitude to self-regulation, strategic dieting behaviour and avoidance of fattening foods. The flexible control subscale (TFEQ-FC) and rigid control subscale (TFEQ-RC) consist of seven questions each (scores ranging from 0 to 7) (Westenhoefer *et al*. 1999). Flexible control (also referred to as consistent restraint) reflects a more gradual approach towards eating and dieting. Foods like sweets and treats or fattening foods are eaten but in smaller quantities, without feelings of guilt. Rigid control (or inconsistent restraint) is characterized by a dichotomous (all-or-nothing) approach towards eating and dieting (Westenhoefer *et al*. 1999). TFEQ-FC has been associated with the ability to control body weight whereas TFEQ-RC has been associated with a lack of control over body weight, bulimia diagnosis, weight fluctuations and body dissatisfaction (Shearin *et al*. 1994). The strategic dieting behaviour subscale consists of four questions (score ranging from 0 to 4) reflecting specific behaviours employed to control weight (e.g. "deliberately taking small helpings"). The attitude to self-regulation (of eating) subscale consists of five questions (score ranging from 0 to 5) which indicate the subjects' general view on dietary intake and weight control. The avoidance of fattening foods subscale consists of four questions (score ranging from 0 to 4), which are concerned with deliberate efforts to decrease fat content in the diet (e.g. `How likely are you to shop for low calorie foods?'). Higher scores for the latter three subscales reflect higher levels of restraint (Bond *et al*. 2001).

The TFEQ-D scale can be divided into three subscales (Bond *et al*. 2001). Habitual susceptibility to disinhibition consists of five questions (score ranging from 0 to 5) and reflects behaviours occurring when circumstances

could predispose to recurrent disinhibition. Emotional susceptibility to disinhibition consists of three questions (score ranging from 0 to 3) and reflects disinhibition associated with negative affective states. Situational susceptibility to disinhibition consists of five questions (score ranging from 0 to 5) and is initiated by specific environmental cues (Bond *et al*. 2001).

Lastly, the TFEQ-H scale can be divided into two subscales (internal and external locus for hunger) with six questions (score ranging from 0 to 6) in each subscale (Bond *et al*. 2001). Internal locus for hunger reflects the type of hunger that is interpreted and regulated internally while external locus for hunger is triggered by external cues (Bond *et al*. 2001).

The scores computed for all scales are continuous variables ranging from a minimum to a maximum, with higher scores for each Factor respectively denoting higher levels of restrained eating, disinhibited eating and predisposition to hunger (Bas & Donmez 2008).

3.12 Psychological health

3.12.1 Beck Depression Inventory (BDI)

The Beck Depression Inventory (BDI) was developed by Beck *et al*. (1961) to measure the presence and severity of depressive symptoms in adults and adolescents older than 13 years. For the purposes of this study the revised BDI-second edition (BDI-II) that was developed from the original BDI and BDI-IA versions to assess depressive symptoms corresponding to the criteria listed in the *Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (DSM-IV)* (Beck *et al*. 1996), was used. The BDI-II can be used in a community and clinical settings to measure the severity of depression in diagnosed patients or to detect possible depression in normal populations (Beck *et al*. 1996). A good test-retest correlation of 0.93 was reported in an out-patient sample that completed the questionnaire one week apart. Two review articles have reported high internal reliability of the BDI-II in both clinical and non-clinical populations with coefficient alpha's greater than 0.80 (Beck *et al*. 1988, Steer *et al*. 1986). Concurrent and construct validation studies also showed significant correlations between the BDI-II and the BDI-IA version and several other psychological tests, including the Beck Hopelessness Scale, Scale for Suicide Ideation, Beck Anxiety Inventory, Revised Hamilton Psychiatric Rating Scale for Depression and the Revised Hamilton Anxiety Rating Scale (Beck & Steer 1987, Beck *et al*. 1996).

The BDI-II is a self-report questionnaire that consists of 21 questions. For each item on the questionnaire subjects can choose one of four possible statements that best describes their feelings over the past two weeks. A 4-point Likert scale is used to score the responses ranging from zero for the absence of depressive symptom or least depressive statement to three for the most depressive statement. Scores of all 21 items are summed to

obtain a total score between zero and 63. Higher scores reflect more severe depressive symptoms (Beck *et al*. 1996).

3.12.2 General Health Questionnaire (GHQ)

The General Health Questionnaire (GHQ) was developed by Goldberg (1972) to be used as a screening instrument for the diagnoses of possible cases of non-psychotic psychiatric disorders among patients attending medical consultation settings (Goldberg 1972). It is a self-administered questionnaire that differentiates between psychiatric patients as a general class from those who consider themselves to be well. For the purposes of this study the 30-item GHQ (Goldberg *et al*. 1976) that was derived from the original 60-item version, was selected. The questionnaire was originally calibrated on British populations and thus constructed for Caucasian population groups (Goldberg *et al*. 1976). The main shortcoming of the GHQ is its tendency to miss patients with long-standing psychiatric disorders (Goldberg *et al*. 1976). Subjects with a psychiatric illness for 'several years' can obtain a low score on the GHQ because of the nature of the response scale i.e. patients may select the 'same as usual' option for most questions and therefore not obtain a high score, despite having a psychiatric disorder (Goldberg *et al*. 1976). This was not considered a limitation for this study, as potential participants with a self-reported psychiatric disorder were excluded from the study.

For each of the 30 items on the GHQ the respondent is asked to compare his/her recent state with his/her usual state by choosing one of four possible answers: 'not at all' (score=0), 'same as usual' (score=0), 'rather more than usual' (score=1) and 'much more than usual' (score=1). A total score between zero and 30 can be obtained. A high score is indicative of poorer mental health. A higher score is also associated with a greater inability to carry out one's normal 'healthy' functions (Goldberg 1972).

3.12.3 Rosenberg Self-Esteem Scale (RSES)

The Rosenberg Self-Esteem Scale (RSES) was developed by Rosenberg (1965) to measure global feelings of self-worth or self-acceptance. The RSES has good reliability with test-retest correlations of 0.82 after a 1-week interval (Fleming & Courtney 1984), 0.85 after a 2-week interval (Silber & Tippett 1965), and coefficient alpha's ranging from 0.75 (Dobson *et al*. 1979) to 0.88 (Fleming & Courtney 1984). Validation studies also showed significant positive correlations between the RSES and measures of confidence and popularity (Lorr & Wunderlich 1986), academic self-concept (Reynolds 1988), general self-regard, social confidence, and school abilities and negative correlations with measures of anxiety and depression (Fleming & Courtney 1984).

The RSES is a self-report questionnaire that consists of 10 items. For each item on the questionnaire the subject can choose one of four possible answers that best describes their present and recent feelings. A 4-point Likert scale is used to score the questions as follows: Strongly disagree (score=1), agree (score=2), disagree (score=3)

and strongly disagree (score=4). A total score between 10 and 40 can be obtained. Higher scores reflect a higher self-esteem (Rosenberg 1965).

3.13 Monitoring of perceived compliance

For the purposes of this study three questions (Figure 3.5) were developed to measure perceived compliance to the three components of the intervention programme (diet, physical activity and behavioural/psychological components) throughout the 16 weeks of the interactive part of the programme.

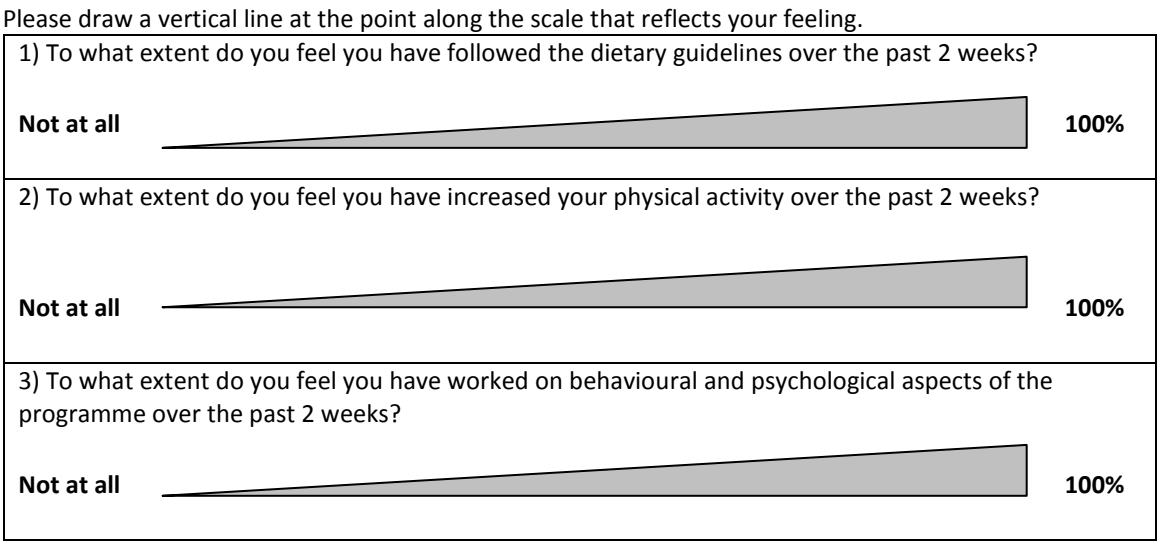

Figure 3.2: Tool for assessment of perceived compliance with dietary, physical activity and behavioural components of the programme

According to Weiten (2001) perceived compliance is a feeling or an attitude and for that reason it would be most appropriately measured using a graduated scale that can be translated into a numerical score. This method is used extensively in psychological research and was deemed appropriate for the purposes of this study (Weiten 2001). It is important to bear in mind that compliance with a weight loss programme leads to weight loss, and that perceived compliance should thus be assessed before weight is measured at a session to ensure that this measure is not influenced by the outcome of the weighing session (Saelens & McGrath, 2003).

Perceived compliance in this study was assessed every two weeks before weight was measured. For these purposes subjects marked their perceived compliance to each of the three components of the programme on a continuous rating scale of 10 cm (Figure 3.2). Perceived compliance was quantified by measuring the point where the mark was made on the scale and assigning a score out of 10 for each subject. The scores collected every two weeks were added and the average score out of 10 for perceived compliance to each component of

the programme was calculated. These three scores were then added to calculate a total perceived compliance score out of 10.

3.14 Additional questions

A self-administered questionnaire (Addendum C) developed for the purposes of the cross-sectional study, was used to obtain information on socio-demographic variables such as age, gender, marital status, home language, highest education levels and number of people with living with. The questionnaire also contained questions on medication use, personal and family weight history, including perception of weight during childhood, adolescence and young adulthood as well as perception of previous and current weight of parents. For the development of this questionnaire a theoretical framework was constructed after which the core concepts to be covered in the questionnaire were finalized. Subsequently the questions were developed and a draft questionnaire was compiled, which was reviewed by a panel of experts and pilot tested on a group of overweight/obese subjects. These steps were followed to ensure construct, content and face validity of the final questionnaire.

3.15 Statistical analyses

Data were entered in Microsoft Excel spreadsheets, transferred to Statistica and cleaned. The different questionnaire scores and BMI indices were computed and values for of MetS traits were categorized according to the indicated diagnostic cut-offs. Data were then analyzed using Statistica computer software (Statistica version 9, Statsoft 2009). The candidate performed all descriptive statistics, Chi-square tests, Independent samples t-tests, One-way ANOVAs and linear models involving the genotype, recessive and dominant genetic models using Statistica. All other statistical analyses involving linear models (with main and interaction effects) for the genotype and additive allelic variables were executed by Prof Lize van der Merwe, Biostatistician at the Medical Research Council of South Africa, on the statistical programme R (from www.r-project.org).

3.15.1 Cross-sectional data (Chapter 4)

Hypotheses

The following primary and secondary hypotheses were formulated for the statistical analyses of the cross-sectional data based on either a physiological explanation for the association or previously reported associations:

Primary null hypothesis:

There is no association between genotype[5] and BMI.

[5] genotype refers to the genotype of the following seven polymorphisms: *FABP2* Ala54Thr, *INSIG2* rs7566605, *FTO* rs1421085, *FTO* rs17817449, *ADRB3* Trp64Arg, *ADRB2* Arg16Gly and *GNB3* C825T.

Secondary null hypotheses:

- There is no association between BMI and health indicators (perceived weight history, MetS prevalence, MetS traits and psychological health).
- There is no association between BMI and lifestyle indicators (physical activity, dietary intake and eating behaviour).
- There is no association between genotype and perceived weight history.
- There is no association between genotype and MetS traits or MetS prevalence.
- The interaction between genotype and BMI has no effect on MetS traits or MetS prevalence.
- There is no association between dietary intake and genotype.
- The interaction between dietary intake and genotype has no effect on BMI.
- There is no association between physical activity and genotype.
- The interaction between physical activity and genotype has no effect on BMI.
- There is no association between eating behaviour and the *FTO* rs1421085, *FTO* rs17817449 and *GNB3* C825T polymorphisms.
- The interaction between eating behaviour and the *FTO* rs1421085, *FTO* rs17817449 and *GNB3* C825T polymorphisms has no effect on BMI.
- There is no association between psychological health and the *GNB3* C825T polymorphism.
- The interaction between psychological health and the *GNB3* C825T polymorphism has no effect on BMI.

To investigate the association between genotype, lifestyle, psychological health and BMI that have not been reported on before or for which a physiological explanation is not known, the following exploratory hypotheses were formulated:

Exploratory null hypotheses:

- There is no association between eating behaviour and the *FABP2* Ala54Thr, *INSIG2* rs7566605, *ADRB3* Trp64Arg and *ADRB2* Arg16Gly polymorphisms.
- The interaction between eating behaviour and the *FABP2* Ala54Thr, *INSIG2* rs7566605, *ADRB3* Trp64Arg and *ADRB2* Arg16Gly polymorphisms has no effect on BMI.
- There is no association between psychological health and the *FABP2* Ala54Thr, *INSIG2* rs7566605, *FTO* rs1421085, *FTO* rs17817449, *ADRB3* Trp64Arg and *ADRB2* Arg16Gly polymorphisms.
- The interaction between psychological health and the *FABP2* Ala54Thr, *INSIG2* rs7566605, *FTO* rs1421085, *FTO* rs17817449, *ADRB3* Trp64Arg and *ADRB2* Arg16Gly polymorphisms has no effect on BMI.

Statistical analyses

For descriptive purposes, means and standard deviations (Mean±SD) were calculated for continuous variables and frequencies for categorical variables. To determine the difference in the mean BMI between the response categories for socio-demographic, perceived weight and MetS variables, the Independent Sample t-test was used in the case of two response categories and the One-way ANOVA test followed by the Tukey post hoc test in

the case of three or more response categories. Independent Sample t-tests were also used to determine the difference in the means of all other continuous variables between male and females. BMI did not differ significantly between females and males. However, as significant differences were detected in a number of other variables, all cross-sectional data was consequently adjusted for gender.

To investigate the association between each MetS trait, lifestyle (Baecke physical activity questionnaire indices, energy-dense food groups and TFEQ scales and subscales) and psychological health (GHQ, BDI and RSES) variables and BMI, linear models were constructed and the main effect sizes were calculated. The main effect size can be interpreted as the average change in BMI corresponding to a one-unit increase in the score of a lifestyle or psychological health variable. For the MetS traits the inverse of the main effect sizes were interpreted as the average change in the MetS trait corresponding to a one-unit increase in BMI (1 kg/m^2).

Genotype distributions were tested for Hardy Weinberg equilibrium using the exact test. Linear models were used to compare the mean BMI between the genotype groups (categorised as the homozygous wild-type, heterozygous and homozygous mutant genotypes) and the additive allelic variable (numerical coded as 0, 1 and 2 minor alleles) for each polymorphism.

To determine the differences between the genotype groups for responses to categories of perceived weight history as well as diagnoses with MetS or MetS traits (Yes/No), cross-tabulations were constructed with genotype category as classification variable. The Pearson's Chi Square statistic was used to test for significant differences in group profiles.

The mean values for MetS trait and scores for lifestyle and psychological health variables were compared between the three genotype groups as well as the dominant (homozygous wild-type genotype vs. mutant allele carriers) and recessive genotype models (wild-type allele carriers vs. homozygous mutant genotype) of each polymorphism using unadjusted and gender-adjusted linear models. For these analyses **only the significant differences are reported** in the tables in the results section. When both unadjusted and gender-adjusted linear models for an association were significant, only the gender-adjusted results are reported. However, in cases of a discrepancy between unadjusted and gender-adjusted results (i.e. one is significant and the other non-significant), both p-values are reported in the tables in the results section.

Gender-adjusted linear models were also used to determine the effect of the interaction between each MetS, lifestyle and psychological health variable and 1) the genotype groups (categorized as wild-type, heterozygous and mutant genotypes) on BMI; and 2) the additive allelic variable (numerically coded as 0, 1 and 2 minor alleles) on BMI. In all cases where significant p-values for the effect of the interaction between **genotype groups** with a specific variable on BMI was found, a significant effect for the **additive allelic variable** interaction with the variable was also found. Therefore, the interaction effect size for the interaction between genotype and the

mentioned variables on BMI is not presented in the results section as it can be derived from the interaction effect size for the additive allelic variable interaction that is presented in the results section.

To visualize significant results, a plot depicting each of the significant additive allelic interactions with a specified variable (x-axes) on BMI (y-axes) was drawn. As gender was not significant when included in the models showing significant interactions, separate plots for males and females were not drawn, but rather the plots representing the total sample and thus unadjusted values. This means that the results were averaged over the genders with the exception of one of the interaction results that were only significant when data were adjusted for gender and the plot (Figure 4.4) depicting this result was thus drawn for a woman. On all plots the symbols represent individual observed values and the regression lines show the expected relationships for each genotype of the polymorphism under investigation. The following symbols were used to identify each genotype group: wild-type (), heterozygous () and mutant genotype (). The plots reflect the modelled rate of change in a specified variable in response to a change in BMI for each genotype group. A significant additive allelic interaction means that the slopes (e.g. increase in BMI corresponding to a one-unit increase/decrease in the MetS, lifestyle and psychological health variable) of the regression lines of each genotype group differ significantly from each other. A positive interaction effect size indicates that the slope of the regression line for the heterozygotes is larger than for subjects with no minor alleles and the slope for the subjects with two minor alleles is even larger. As the minor allele was not always the mutant allele for the investigated polymorphisms, the minor allele is indicated below each plot in the results section. A positive additive allelic interaction effect size of for example 4 would mean that the addition of one minor allele (thus heterozygote) would result in a 4-unit **increase** in the slope of BMI for each one-unit increase in a MetS, lifestyle and psychological health variable when compared to subjects with no minor alleles. Therefore, two minor alleles would result in an 8-unit (2X4=8) increase in the slope of BMI for each one-unit increase in a specified variable compared to subjects with no minor alleles. A negative interaction effect size indicates that the slope of the line for the heterozygotes is smaller than for subjects with no minor alleles and the slope for the subjects with two minor alleles is even smaller. A negative additive allelic interaction effect size of -4 would mean that the addition of one minor allele (thus heterozygote) would result in a 4-unit **decrease** in the slope of BMI for each one-unit increase in a specified variable when compared to subjects with no minor alleles. It is thus important to note that the positive or negative designation of the interaction effect sizes is only an indication of whether the slopes increase or decrease with the number of minor alleles. Therefore, to interpret the association between BMI and a specified variable for a genotype group (thus whether this association is in a positive or negative direction) the plot (and not the designation of the interaction effect size) must be used. The interpretation of the plots in the results section focuses on the genotype group that depicts the typically expected association between BMI and the specified variable (thus when genotype groups are not considered) and for which the most pronounced regression line slope was found.

Gender-adjusted linear models were also used to test for the interaction between the *FTO* haplotype and each MetS, lifestyle and psychological health variable on BMI.

3.15.2 Intervention data (Chapter 5)

Hypotheses

Primary null hypothesis:

- There is no association between genotype[6] and weight loss outcomes in overweight/obese subjects after following a conservative weight loss programme for four, eight, 12, 16 and 24 weeks.

Secondary null hypotheses:

- There is no change in the weight of overweight/obese subject after following the conservative weight loss programme for a period of 24 weeks.
- There are no changes in selected psychological health and lifestyle indicators that were the focus of strategies included in the weight loss programme from baseline to 16 week follow-up.
- The interaction between genotype and change in physical activity levels has no effect on change in BMI over the 24 week intervention period.
- The interaction between the *FTO* rs1421085, *FTO* rs17817449 and *GNB3* C825T polymorphisms and change in eating behaviours has no effect on change in BMI over the 24 week intervention period.
- The interaction between the *GNB3* C825T polymorphism and change in psychological health has no effect on change in BMI over the 24 week intervention period.

Exploratory null hypotheses:

- The interaction between the *FABP2* Ala54Thr, *INSIG2* rs7566605, *ADRB3* Trp64Arg and *ADRB2* Arg16Gly polymorphisms and change in eating behaviours has no effect on change in BMI over the 24 week intervention period.
- The interaction between the *FABP2* Ala54Thr, *INSIG2* rs7566605, *FTO* rs1421085, *FTO* rs17817449, *ADRB3* Trp64Arg and *ADRB2* Arg16Gly polymorphisms and change in psychological health has no effect on change in BMI over the 24 week intervention period.

Statistical analyses

For descriptive purposes, Mean±SD were calculated for continuous variables and frequencies for categorical variables. To determine the difference in the mean BMI between the response categories for each socio-demographic variable the Independent Sample t-test was used in the case of two response categories and the One-way ANOVA test in the case of three or more response categories. To determine the difference in the mean weight, BMI, age or scores of all lifestyle and psychological health variables between subjects who volunteered to participate in the weight loss programme and those who only completed the cross-sectional assessments, the Independent Samples t-test was used. To test for associations between age or compliance and baseline weight/BMI and weight/BMI change over the six month period a Pearson's correlation matrix was constructed.

[6] genotype refers to the genotype of the following seven polymorphisms: *FABP2* Ala54Thr, *INSIG2* rs7566605, *FTO* rs1421085, *FTO* rs17817449, *ADRB3* Trp64Arg, *ADRB2* Arg16Gly and *GNB3* C825T.

Differences in mean baseline weight/BMI and weight/BMI change over the six month period between males and females were determined with an Independent samples t-test.

Linear models were used to determine the mean (SE) difference between baseline and 24-week follow-up weight and BMI. Linear mixed-effects models with weeks as fixed effect and individual as random effect were constructed to determine the difference in weight and BMI from baseline to each follow-up point (every two weeks on the programme). For these purposes longitudinal time-trajectories that make it possible to incorporate the correlation between observations on the same individual at different time-points, were used. Linear models with the 16-week follow-up variable as a function of the baseline variable were constructed to determine the mean difference and standard error (SE) of this difference between baseline and 16-weeks follow-up scores for lifestyle and psychological health variables.

As females lost significantly more weight than males and total compliance was significantly associated with BMI and weight loss (see Chapter 5, Table 5.3), the linear models that were constructed to investigate the effect of genotype alone or in combination with other variables on weight or BMI change over the six month intervention period (Δ BMI), were adjusted for gender and compliance. Linear mixed-effects models of genotype on Δ BMI as fixed effect and individual as random effect (to adjust for correlations between observations on the same individual) were constructed to determine the genotype and additive allelic effects on time trajectories (change in weight and BMI) for different time-periods from the start of the intervention. Significant genotype or additive allelic effects were depicted in plots with modelled weight against time (in weeks) on the weight loss programme. Linear models were also used to investigate the effect of the interaction between genotype and change in lifestyle or psychological health variables over the four month period (Δ variable) on Δ BMI. Only the signficant additive allelic interaction effects are illustrated in plots in the results section. These modelled graphs were constructed for a female with an average compliance of 4.9. In these graphs, Δ BMI refers to baseline minus follow-up BMI, thus a positive difference on the y-axes should be interpreted as a BMI loss (i.e. baseline BMI is more than follow-up BMI). The Δ variable also reflects the baseline minus follow-up scores of the specific questionnaire depicted on the x-axes. Therefore, a positive value on the x-axes reflects a decrease in the specific questionnaire score from baseline to follow-up.

Linear models were also used to investigate the effect of all gene-gene combinations on BMI change over the 24 week intervention period.

In all statistical analyses a p-value of <0.05 was designated to be statistically significant. However, it must be borne in mind that with multiple comparisons as performed in this thesis, a false significant p-value (of <0.05) can be expected to occur once in every 20 tests. To overcome this problem Bonferroni adjustments and stringent p-values can be used. However, Perneger (1998) argued that such adjustments have limited value in medical research. This author points to the fact that the significant result of a test (for example a positive association between BMI and cholesterol) will be considered true if it was performed together with a small

number of other tests (<20). However, the same result will be obtained when it is performed along with 50 other tests, which does not justify the exclusion of the result just because the number of tests has increased. Perneger (1998) therefore recommends that the results from all tests are important to consider and that each significant p-value should rather be interpreted with caution, taking into account the plausibility of the finding. This approach was followed in this thesis.

3.16 Ethical issues

Ethical permission for this research was obtained from the Health Research Ethics Committee of Stellenbosch University and the Human Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town. The research was conducted according to international and locally accepted ethical guidelines for research, namely the Declaration of Helsinki, Guidelines on Ethics for Medical and Genetic Research of the Medical Research Council of South Africa. After being thoroughly informed, each volunteer signed a consent form (Addendum B). Subjects were assured that data would be handled confidentially and only published within group context.

Chapter 4

Cross-sectional study

Results, discussion and conclusions

4.1 Results

Socio-demographic and weight profile of the cross-sectional sample

The subjects had a mean±SD age of 32.9±4.4 years, weight of 99.9±20.1 kg, height of 1.68±0.08 m and BMI of 35.2±6.6 kg/m^2. In this sample 21.8% of subjects were overweight (BMI of 27-29.9 kg/m^2), 33.1% were obese class I (BMI of 30-34.9 kg/m^2), 24.1% were obese class II (BMI of 35-39.9 kg/m^2) and 21.0% were obese class III (BMI ≥ 40 kg/m^2). Most were female, married, Afrikaans speaking, had a tertiary qualification and lived with their partners and children (Table 4.1). There were no significant differences in mean BMI between the response categories of the socio-demographic variables. Two subjects out of 133 (0.02%) used medication for hypertension, three subjects (0.02%) used statins for high LDL-cholesterol, seven subjects (0.05%) were on oral contraceptives while 11 (0.08%) used anti-depressants.

Table 4.1: Socio-demographic profile of overweight/ obese subjects (n=133)

	n	%	BMI (kg/m^2) mean±SD	p-value
Gender				
Female	112	84.2	35.3±6.9	0.818[†]
Male	21	15.8	34.9±4.5	
Marital status				
Married / living together	86	64.7	35.1±6.3	0.703[†]
Unmarried (including separated/ divorced)	47	35.3	35.5±7.1	
Home language				
Afrikaans	120	90.2	35.2±6.5	0.729[†]
English	13	9.8	35.8±7.2	
Level of education				
Completed Grade 10 or Matric	43	32.3	35.1±7.3	0.887[†]
Tertiary qualification	90	67.7	35.3±6.2	
Living				
alone	22	16.5	37.4±8.0	0.156[§]
with friends/ parents	20	15.0	33.7±6.0	
with a partner	30	22.6	33.8±5.6	
with a partner and child(ren)	61	45.9	35.7±6.5	

[†] Independent Samples t-test

[§] One-way ANOVA

Genotype and allele frequencies

The genotype and allele frequencies for the polymorphisms investigated in the study sample are presented in Table 4.2.

Table 4.2: Genotype and allele frequencies of overweight/obese Caucasian subjects and association with BMI.

Gene polymorphism	Genotype frequencies			Association with BMI			Allele frequencies		HWE
	Base pair[‡]	n*	%	BMI mean±SD	genotype[†] p-value	additive[§] p-value		%	p-value[Γ]
FABP2 Ala54Thr	GG	52	46.4	35.4±6.6	0.4937	0.4421	G-allele	65.6	0.1410
	GA	43	38.4	35.8±7.1			A-allele	34.4	
	AA	17	15.2	33.5±6.7					
INSIG2 rs7566605	GG	51	44.4	35.0±6.6	0.8382	0.5530	G-allele	68.3	0.3880
	GC	55	47.8	35.5±6.9			C-allele	31.7	
	CC	9	7.8	36.2±6.6					
FTO rs1421085	TT	21	20.8	34.5±7.3	0.1715	0.6764	T-allele	48.0	0.4284
	TC	55	54.5	36.6±7.0			C-allele	52.0	
	CC	25	24.7	33.6±5.4					
FTO rs17817449	TT	31	29.5	35.9±7.0	0.8642	0.8931	T-allele	44.8	**0.0001**
	TG	32	30.5	34.9±6.8			G-allele	55.2	
	GG	42	40.0	36.0±7.2					
ADRB3 Trp64Arg	TT	84	90.3	35.8±7.0	0.2410	0.2410	T-allele	95.2	1.0000
	TC	9	9.7	33.3±3.5			C-allele	4.8	
	CC	0	0	-					
ADRB2 Arg16Gly	AA	19	17.9	35.5±6.8	0.8129	0.6703	A-allele	42.0	1.0000
	AG	51	48.1	35.1±7.1			G-allele	58.0	
	GG	36	34.0	36.1±6.1					
GNB3 C825T	CC	52	48.6	36.1±7.6	0.6332	0.4004	C-allele	65.4	**0.0098**
	CT	36	33.6	34.8±5.8			T-allele	34.6	
	TT	19	17.8	34.9±5.6					

*n varies due to missing values

[‡]Ascending order: wild-type then heterozygous and mutant genotypes.

[†]Gender-adjusted linear model testing the association between BMI and the genotype groups (categorised as the homozygous wild-type, heterozygous and homozygous mutant genotypes).

[§]Gender-adjusted linear model testing the association between BMI and the additive allelic variable (numerically coded as 0, 1 and 2 minor alleles).

[Γ]Exact test; HWE = Hardy-Weinberg equilibrium

The frequency of the homozygous mutant (GG) genotype of the *FTO* rs17817449 polymorphism was higher than that of the homozygous wild-type or heterozygous genotypes. The frequency of the heterozygous genotype was higher than the homozygous wild-type or homozygous mutant genotypes of the *INSIG2* rs7566605, *ADRB2* Arg16Gly and *FTO* rs1421085 polymorphisms. No homozygous mutant (CC) genotype was found in this sample for the *ADRB3* Trp64Arg polymorphism. The frequencies of the mutant alleles were higher than the wild-type alleles of the *ADRB2* Arg16Gly, *FTO* rs1421085 and rs17817449 polymorphisms. Although the genotype frequencies of the *GNB3* C825T and *FTO* rs17817449

polymorphisms were not in HWE, it must be borne in mind that all subjects were overweight/obese and not representative of a random population. This could provide an explanation for the deviations from HWE.

It is evident from Table 4.2 that no associations between BMI and genotype or additive allelic groups were found.

Weight related perceptions

Only two thirds (62.1%) of subjects perceived their weight as normal during their childhood years while a third already perceived themselves as overweight (Table 4.3). Prevalence of "perceiving weight as normal" decreased during adolescence (50.8%) and almost two thirds perceived themselves as overweight or obese (65.9%) during young adulthood (ages 20 to 25 years). The mean current BMI of subjects who perceived their weight as either overweight/obese during childhood, adolescence and young adulthood was significantly higher than those who perceived their weight as normal during these life cycle phases (Table 4.3).

Most subjects felt that their mothers were overweight or obese during their (the subjects') adolescence and that their fathers' weight was normal. However, when subjects started with this study most thought that their parents were either overweight or obese. There were no significant differences between the different response categories for perception of parents' current or previous weight and the subjects' mean current BMI (Table 4.3).

Subjects with the homozygous wild-type Arg16Arg genotype of the *ADRB2* Arg16Gly polymorphism were significantly more likely to perceive themselves as overweight during their childhood years, while those with the heterozygous or homozygous mutant genotypes mostly thought they had a normal weight (Table 4.4). Subjects with the homozygous wild-type or mutant genotypes of the *GNB3* C825T polymorphism were significantly more likely to perceive their weight as normal during their childhood years than the heterozygotes. During their adolescent years, only those with the homozygous mutant *GNB3* genotype were significantly more likely to perceive their weight as normal while those with the homozygous wild-type and heterozygous genotypes mostly thought they were overweight at the time. However, these associations between perceived weight and the *GNB3* C825T polymorphism disappeared in young adulthood. Subjects with the homozygous mutant CC genotype of the *FTO* rs1421085 polymorphism were significantly more likely to perceive their weight as normal in adolescence, while the TT and TC genotypes mostly thought they were overweight. There were no significant associations between the subjects' genotypes for all polymorphisms tested and perception of parents' current or previous weight (results not presented in a Table).

Table 4.3: Weight related perceptions and association with mean±SD BMI.

Perceived weight categories	n	%	Collapsed perceived weight categories*	%	Current BMI mean±SD	p-value[§]
Perceived weight during childhood						
Underweight	3	2.3				
Normal weight	82	62.1	Normal	62.4	33.8±5.5	**0.0003**
Overweight	44	33.3	Overweight	35.6	38.0±7.5	
Obese	3	2.3				
Perceived weight during adolescence						
Underweight	1	0.8				
Normal weight	66	50.0	Normal weight	50.8	34.1±6.4	**0.0322**
Overweight	61	46.2	Overweight	49.2	36.5±6.6	
Obese	4	3.0				
Perceived weight when 20 to 25 years old						
Underweight	1	0.8				
Normal weight	44	33.3	Normal weight	34.1	32.6±5.7[a]	**<0.0001**
Overweight	70	53.0	Overweight	53.0	35.8±6.5[b]	
Obese	17	12.9	Obese	12.9	40.3±5.8[c]	
Perception of mother's weight when subject was an adolescent						
Underweight	2	1.5				
Normal weight	60	45.4	Normal weight	46.9	35.6±7.5	0.4400
Overweight	62	47.0	Overweight	53.1	34.7±5.7	
Obese	8	6.1				
Perception of father's weight when subject was an adolescent						
Underweight	1	0.8				
Normal weight	80	60.6	Normal weight	62.3	35.4±6.7	0.9130
Overweight	42	31.8	Overweight	37.7	35.2±6.5	
Obese	7	5.3				
Diseased/ did not know him	2	1.5				
Perception of mother's current weight						
Underweight	5	3.8				
Normal weight	47	35.6	Normal weight	41.9	35.6±7.6	0.6430
Overweight	58	43.9	Overweight	58.1	35.0±5.9	
Obese	14	10.6				
Diseased/ did not know her	8	6.1				
Perception of father's current weight						
Underweight	1	0.8				
Normal weight	49	37.1	Normal weight	46.3	34.8±6.6	0.6340
Overweight	47	35.6	Overweight	53.7	35.5±7.0	
Obese	11	8.3				
Diseased/ did not know him	24	18.2				

*Underweight and normal weight categories were collapsed as "normal weight", while overweight and obese categories were collapsed as "overweight" except for "perceived weight when 20 to 25 years old", where only underweight and normal weight categories were combined.

[§] Gender-adjusted linear models; in the case of 3 categories the means with the same letter do not differ significantly using the Tukey post-hoc test.

Table 4.4: Association between weight related perceptions and genotypes.

Life cycle stages	Polymorphisms	Perception of weight	Column % of genotypes × perception			
			Wild-type	Heterozygous	Mutant	p-value*
Perception of own childhood weight	*ADRB2* Arg16Gly	Normal	42.1	74.0	61.1	**0.0442**
		Overweight	57.9	26.0	38.9	
	GNB3 C825T	Normal	76.9	48.6	73.7	**0.0177**
		Overweight	23.1	51.4	26.3	
Perception of own adolescent weight	*FTO* rs1421085	Normal	33.3	47.3	76.0	**0.0102**
		Overweight	66.7	52.7	24.0	

*Pearson Chi square test

Non-significant differences not included in table

Metabolic Syndrome (MetS)

MetS was diagnosed in 46.8% of subjects using the ATP NCEP III criteria (Table 4.5). The most prevalent trait contributing to the diagnoses of MetS was a large waist circumference, followed by a low HDL and high blood pressure. The mean waist circumference, HDL and blood pressure were in the ranges set for the diagnosis of MetS, while the mean glucose and triglycerides were below these cut-offs. Subjects who were diagnosed with MetS as well as those who met the respective diagnostic cut-offs for waist circumference, HDL and blood pressure had a significantly higher mean BMI than subjects who were not diagnosed with MetS or who did not meet the respective cut-off values (Table 4.5). Only 4% of the subjects met none of the diagnostic cut-offs set for each MetS trait, while 22.7% met one, 26.7% met two, 34.7% met three, 10.7% met four and 1.3% met all the cut-off values. This was also reflected in the fact that the subjects met the diagnostic cut-offs for a mean±SD of 2.3±1.1 MetS traits (data not presented in a Table).

Table 4.5: Mean±SD for MetS traits (ATP NCEP III criteria), % subjects who met the relevant diagnostic cut-offs as well as association with BMI.

MetS traits		n[§]	Mean±SD	Cut-offs for MetS NCEP ATP III criteria	MetS diagnosed Yes/No	%	BMI mean±SD	p-value[†]
Triglycerides (mmol/L)		88	1.17±0.60	≥1.69 mmol/L	Yes No	19.3 80.7	36.2±5.8 35.6±7.0	0.7830
Waist circumference (cm)	All	84	108.1±14.2	Women: >88 cm Men: >102 cm	Yes No	92.9 7.1	36.4±6.7 28.2±1.3	**0.0044**
	♀	72	106.6±13.9					
	♂	12	117.6±12.0					
Blood pressure (mmHg)		84		≥130/85 mmHg or currently treated with hypertension medication	Yes No	41.7 58.3	38.2±6.7 34.0±6.4	**0.0048**
Systolic		84	132.4±17.1					
Diastolic		84	88.0±13.8					
Glucose (mmol/L)		88	5.06±0.55	≥6.1 mmol/L	Yes No	6.8 93.2	37.9±9.9 35.6±6.5	0.4067
HDL (mmol/L)	All	84	0.97±0.19	Women: <1.24 mmol/L Men: <1.04 mmol/L	Yes No	67.9 32.1	36.7±6.7 33.2±6.2	**0.0235**
	♀	72	1.14±0.31					
	♂	12	1.11±0.30					
MetS diagnosed		77	-	3 or more of above traits	Yes No	46.8 53.2	37.8±6.4 33.3±6.6	**0.0032**

[†] Gender-adjusted linear model

[§] n varies due to missing values

MetS = metabolic syndrome; ♀ = women; ♂ = men

Significant associations between mean values for different MetS traits and the *GNB3* C825T and *INSIG2* rs7566605 polymorphisms were found (Table 4.6). Subjects with the mutant TT genotype of the *GNB3* C825T polymorphism had significantly higher mean glucose and triglyceride levels, while those with the mutant CC genotype of the *INSIG2* rs7566605 polymorphism had significantly lower HDL levels. Subjects with the latter two mutant genotypes were also significantly more inclined to meet the criteria of a higher number of traits for the diagnosis of MetS. Furthermore, subjects with the mutant TT genotype of the *GNB3* C825T polymorphism were more likely (p=0.0322) to be diagnosed with MetS compared to those with wild-type and heterozygous genotypes of this polymorphism (Figure 4.1). No significant associations were found between the polymorphisms investigated in this study and the percentage subjects who met the diagnostic cut-offs for each of the five MetS traits individually (results not presented in a Table).

Table 4.6: Significant associations between MetS traits and genotype.

MetS trait	Polymorphisms	Genotypes	n*	mean±SD	p-value[§]
Triglycerides (mmol/L)	*GNB3* C825T	C-allele carriers	62	1.1±0.6	**0.0447**
		TT	10	1.5±0.6	
Glucose (mmol/L)	*GNB3* C825T	CC	34	5.0±0.5[a]	**0.0322**
		CT	29	5.0±0.6[a,b]	
		TT	9	5.5±0.8[b]	
	GNB3 C825T	C-allele carriers	63	5.0±0.5	**0.0090**
		TT	9	5.5±0.8	
HDL (mmol/L)	*INSIG2* rs7566605	GG	33	1.1±0.3[a,b]	**0.0343**
		CG	34	1.2±0.3[a]	
		CC	7	0.9±0.2[b]	
	INSIG2 rs7566605	G-allele carriers	67	1.2±0.3	**0.0164**
		CC	7	0.9±0.2	
Number of MetS traits	*GNB3* C825T	C-allele carriers	55	2.2±1.0	**0.0417**
		TT	9	3.0±1.0	
	INSIG2 rs7566605	G-allele carriers	60	2.2±1.1	**0.0327**
		CC	6	3.2±1.0	

*n vary due to missing values; [§] Gender-adjusted linear models, in the case of 3 categories the means with the same letter do not differ significantly using the Tukey post-hoc test.

Non-significant differences not included in table; MetS = metabolic syndrome

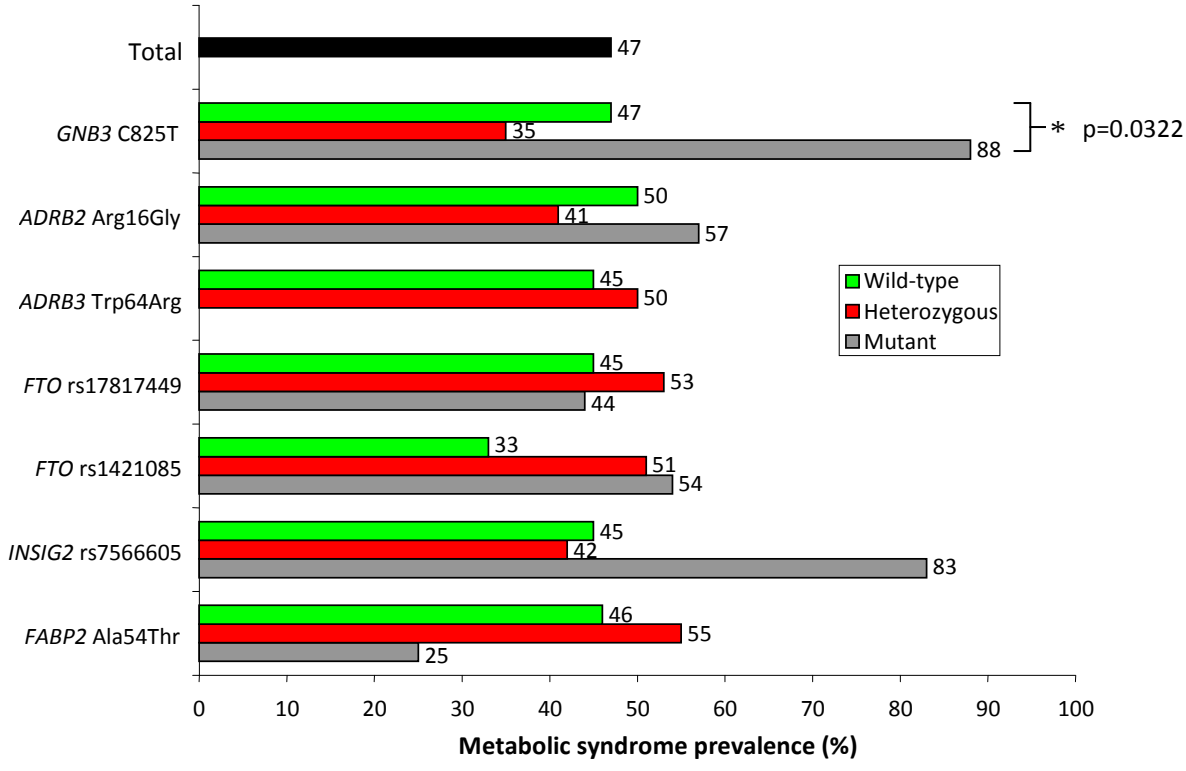

Figure 4.1: Prevalence of MetS in overweight/ obese subjects in the total sample and according to genotypes.

The main effect sizes of the average change in BMI corresponding to a one-unit increase in each MetS trait are presented in Table 4.7. For instance, a one-unit (1 cm) increase in waist circumference was significantly associated with a 0.41 kg/m^2 increase in BMI. Interpreting the inverse of the main effect sizes that would correspond to a one-unit change in BMI, revealed that a one-unit (1 kg/m^2) increase in BMI was significantly associated with a 2.44 cm increase in waist circumference, 4.55 mmHg increase in systolic and 5.56 mmHg increase in diastolic blood pressure and a 0.20 mmol/L decrease in HDL. Furthermore, each 2.4 kg/m^2 increase in BMI was significantly associated with the diagnosis of an additional MetS trait (Table 4.7).

The rate of change in a MetS trait in response to a change in BMI differed significantly between the genotype groups of the *FABP2* Ala54Thr, *FTO* rs1421085 and rs17817449 polymorphisms (Table 4.7). The most pronounced increase in triglycerides in response to an increase in BMI was observed for the wild-type homozygotes of *FABP2* Ala54Thr (Figure 4.2). The most pronounced increase in HDL in response to a decrease in BMI was observed for the homozygous mutant genotypes of the *FTO* rs1421085 (Figure 4.3) and *FTO* rs17817449 (Figure 4.4) polymorphisms. Similarly, the most pronounced increase in HDL in response to an decrease in BMI was observed for the mutant (C-G) *FTO* haplotype compared to the wild-type T-T haplotype of these two *FTO* polymorphisms (effect size = 13.6, p=0.0135, results not included in a Table or Figure). No significant differences were found between the other haplotype combinations.

Table 4.7: The main effect size (the average change in BMI in response to a one-unit increase in each MetS trait) and corresponding p-values, as well as the interaction effect size and corresponding p-values of the interaction between MetS traits and polymorphisms on BMI.

MetS traits	**Effect of MetS traits on BMI**		**Effect of MetS trait × polymorphism on BMI**			
	Main effect size	**p-value***	**Genes and polymorphisms**	**Genotype p-value†**	**Minor allele Interaction effect size**	**Additive p-value§**
Triglyceride (mmol/L)	1.11	0.3701	*FABP2* Ala54Thr	**0.0018**	-6.20	**0.0021**
			INSIG2 rs7566605	0.7193	-1.53	0.3909
			FTO rs1421085	0.4602	1.85	0.3810
			FTO rs17817449	0.1594	-1.33	0.4438
			ADRB3 Trp64Arg	0.4433	-11.17	0.4433
			ADRB2 Arg16Gly	0.8564	-0.87	0.6444
			GNB3 C825T	0.7095	-0.48	0.8062
Waist circumference (cm)	0.41	**<0.0001**	*FABP2* Ala54Thr	0.9499	0.00	0.9639
			INSIG2 rs7566605	0.4873	-0.01	0.9136
			FTO rs1421085	0.6364	-0.04	0.5155
			FTO rs17817449	0.9560	0.01	0.8098
			ADRB3 Trp64Arg	0.7426	-0.09	0.7426
			ADRB2 Arg16Gly	0.4933	0.03	0.5360
			GNB3 C825T	0.6257	0.00	0.9560

Table 4.7: *(continued)*

MetS traits	Effect of MetS traits on BMI		Effect of MetS trait × polymorphism on BMI			
	Main effect size	p-value*	Genes and polymorphisms	Genotype p-value†	Minor allele Interaction effect size	Additive p-value§
Systolic blood pressure (mmHg)	0.22	**<0.0001**	*FABP2* Ala54Thr	0.7649	0.03	0.6316
			INSIG2 rs7566605	0.7972	0.05	0.5258
			FTO rs1421085	0.1959	-0.06	0.2523
			FTO rs17817449	0.6580	-0.01	0.8596
			ADRB3 Trp64Arg	0.7184	0.16	0.7184
			ADRB2 Arg16Gly	0.2847	0.03	0.6026
			GNB3 C825T	0.4807	-0.02	0.7345
Diastolic blood pressure (mmHg)	0.18	**0.0010**	*FABP2* Ala54Thr	0.3518	0.12	0.2230
			INSIG2 rs7566605	0.8432	-0.04	0.6902
			FTO rs1421085	0.7457	0.05	0.5716
			FTO rs17817449	0.5055	-0.03	0.6609
			ADRB3 Trp64Arg	0.5494	0.60	0.5494
			ADRB2 Arg16Gly	0.2986	0.02	0.8181
			GNB3 C825T	0.5784	0.04	0.6435
Glucose (mmol/L)	0.11	0.9319	*FABP2* Ala54Thr	0.5060	-2.87	0.2654
			INSIG2 rs7566605	0.1561	0.89	0.6901
			FTO rs1421085	0.8425	-0.87	0.7049
			FTO rs17817449	0.1343	2.99	0.0626
			ADRB3 Trp64Arg	0.3815	-6.50	0.3815
			ADRB2 Arg16Gly	0.7124	-0.58	0.8043
			GNB3 C825T	0.9986	0.29	0.8748
HDL (mmol/L)	-5.42	**0.0334**	*FABP2* Ala54Thr	0.3485	2.60	0.5663
			INSIG2 rs7566605	0.8745	0.22	0.9630
			FTO rs1421085	0.0587	-10.09	**0.0270**
			FTO rs17817449	0.0800	6.84	**0.0454**
			ADRB3 Trp64Arg	0.7872	2.99	0.7872
			ADRB2 Arg16Gly	0.1022	-3.95	0.3686
			GNB3 C825T	0.9863	0.67	0.8789
Number of MetS traits	2.40	**0.0008**	*FABP2* Ala54Thr	0.3986	-1.85	0.1711
			INSIG2 rs7566605	0.2665	-1.10	0.3663
			FTO rs1421085	0.4793	1.55	0.2351
			FTO rs17817449	0.4026	0.37	0.7221
			ADRB3 Trp64Arg	0.7810	1.40	0.7810
			ADRB2 Arg16Gly	0.4273	-0.67	0.5525
			GNB3 C825T	0.8654	-0.61	0.6273

All statistical models were adjusted for gender.

*Linear model of the main effect of specific MetS traits on BMI.

†Linear model of the interaction between each MetS trait and genotype groups (categorised as wild-type, heterozygous and mutant genotypes) on BMI.

§Linear model of the interaction between each MetS trait and the additive allelic variable (numerically coded as 0, 1 and 2 minor alleles) on BMI.

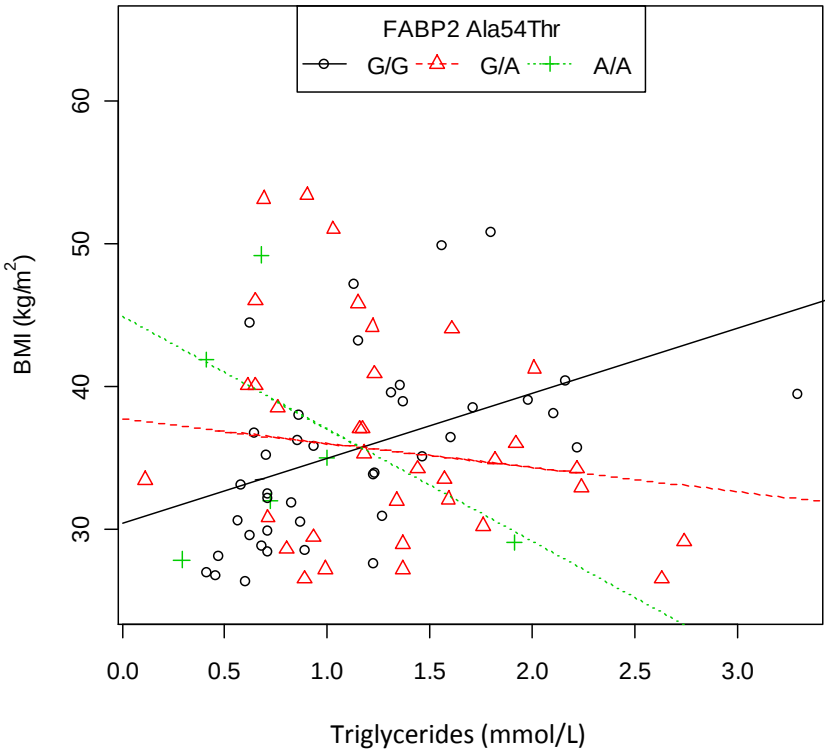

Figure 4.2: Plot of BMI against triglycerides. Symbols represent individual observed values and regression lines show the expected relationships for each genotype of the *FABP2* Ala54Thr polymorphism (minor allele = A).

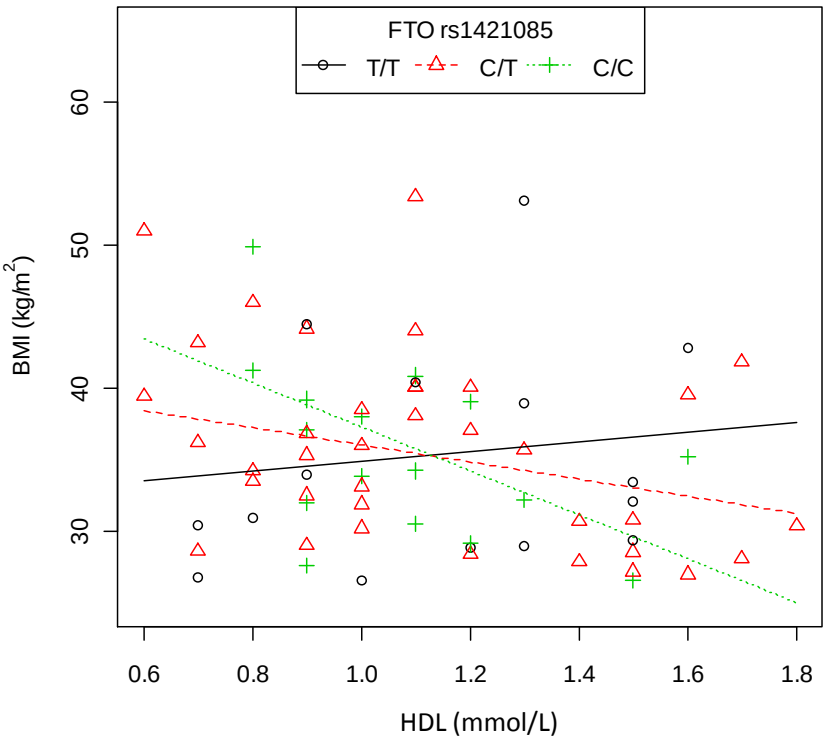

Figure 4.3: Plot of BMI against HDL. Symbols represent individual observed values and regression lines show the expected relationships for each genotype of the *FTO* rs1421085 polymorphism (minor allele = T).

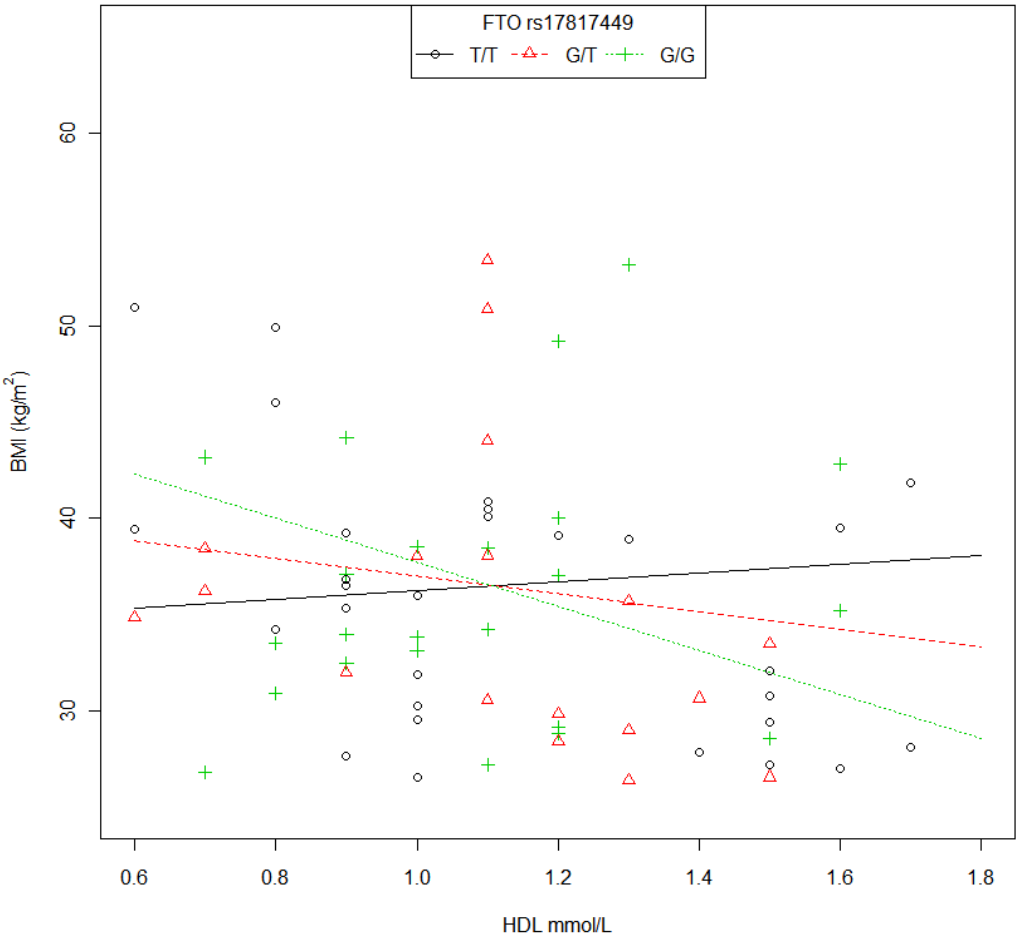

Figure 4.4: Plot of BMI against HDL. Symbols represent individual observed values and regression lines show the expected relationships for a woman for each genotype of the *FTO* rs17817449 polymorphism (minor allele = T).

Physical activity

The mean±SD scores of the work, sport and leisure-time indices are presented in Table 4.8. A one-point increase in the sport index or leisure-time index score was significantly associated with a 2.01 kg/m^2 and 2.43 kg/m^2 decrease in BMI respectively (Table 4.8).

The only significant association found between genotype groups and mean±SD scores for the work, sport and leisure-time indices was for the *INSIG2* rs7566605 polymorphism. The mutant C-allele carriers had a higher mean±SD score of 2.2±0.6 compared to the score (1.9±0.4) of the wild-type GG homozygotes (p= 0.0470) (not presented in a Table).

The rate of change in BMI in response to a change in the leisure-time index score differed significantly between the genotype groups of the *GNB3* C825T and *INSIG2* rs7566605 polymorphisms. There was a more pronounced decrease in BMI in response to an increase in the leisure-time index score for the wild-type homozygotes (CC and GG respectively) of the *GNB3* C825T (Figure 4.5) and *INSIG2* rs7566605 polymorphisms (Figure 4.6) that was not observed for the mutant homozygotes.

Haplotype analysis of the two *FTO* polymorphisms revealed that the most pronounced decrease in BMI in response to an increase in the sport index score was observed for the wild-type T-T haplotype compared to the T-G haplotype (effect size = -0.655, p=0.0469) (results not presented in a Table). No significant differences were found between the other haplotype combinations.

Table 4.8: Mean±SD scores of the three physical activity indices, the main effect size (the average change in BMI in response to a one-unit unit increase in each physical activity index score) and corresponding p-values, as well as the interaction effect size and corresponding p-values of the interaction between physical activity indices and polymorphisms on BMI.

Physical activity (PA) index	PA index score Mean±SD	Effect of PA indices on BMI		Effect of PA index × polymorphism on BMI			
		Main effect size	p-value*	Genes and polymorphisms	Genotype p-value†	Minor allele Interaction effect size	Additive p-value§
Work index	2.4±0.6	1.08	0.3044	*FABP2* Ala54Thr	0.2958	0.56	0.7542
				INSIG2 rs7566605	0.9315	-0.67	0.7460
				FTO rs1421085	0.1716	0.82	0.6785
				FTO rs17817449	0.8756	-0.48	0.7762
				ADRB3 Trp64Arg	0.4121	-5.16	0.4121
				ADRB2.Arg16Gly	0.8871	0.74	0.6845
				GNB3 C825T	0.1569	2.39	0.1266
Sport index	2.0±0.7	-2.10	**0.0194**	*FABP2* Ala54Thr	0.7049	0.20	0.9029
				INSIG2 rs7566605	0.8508	-0.61	0.7218
				FTO rs1421085	0.1424	1.50	0.2896
				FTO rs17817449	0.2774	-1.06	0.3949
				ADRB3 Trp64Arg	0.7812	1.14	0.7812
				ADRB2.Arg16Gly	0.2041	-2.47	0.0903
				GNB3 C825T	0.1688	1.19	0.3705
Leisure-time index	2.1±0.5	-2.43	**0.0233**	*FABP2* Ala54Thr	0.8880	-0.32	0.8428
				INSIG2 rs7566605	0.0797	4.33	**0.0243**
				FTO rs1421085	0.0930	2.00	0.2688
				FTO rs17817449	0.4718	-1.83	0.2169
				ADRB3 Trp64Arg	0.5333	-3.65	0.5333
				ADRB2.Arg16Gly	0.4918	-1.95	0.2470
				GNB3 C825T	**0.0388**	3.24	**0.0447**

All statistical models were adjusted for gender

*Linear model of main effects of the work, sport and leisure-time index scores on BMI.

†Linear model of the interaction between each physical activity index and genotype groups (categorised as wild-type, heterozygous and mutant genotypes) on BMI.

§Linear model of the interaction between each physical activity index and the additive allelic variable (numerically coded as 0, 1 and 2 minor alleles) on BMI.

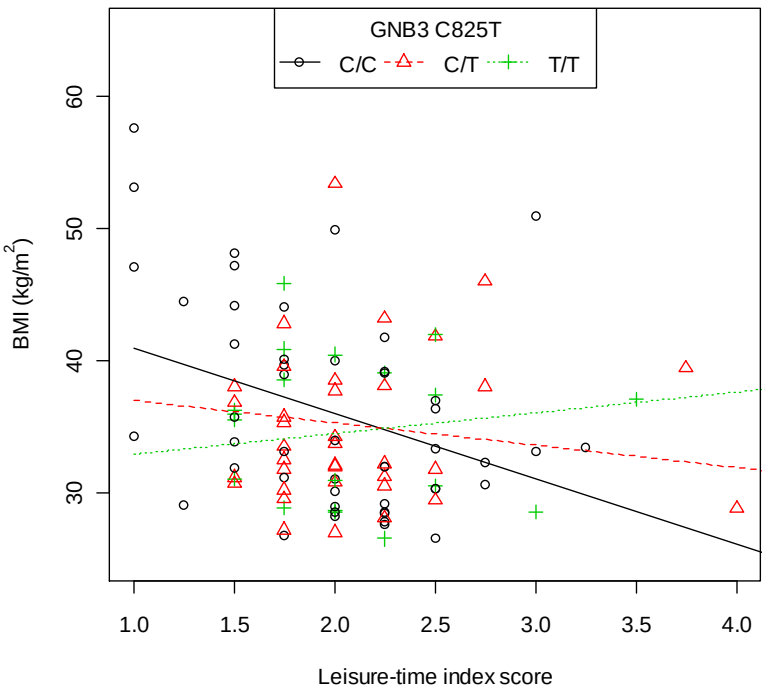

Figure 4.5: Plot of BMI against the leisure-time index score of the Baecke physical activity questionnaire. Symbols represent individual observed values and regression lines show the expected relationships for each genotype of the *GNB3* C825T polymorphism (minor allele = T).

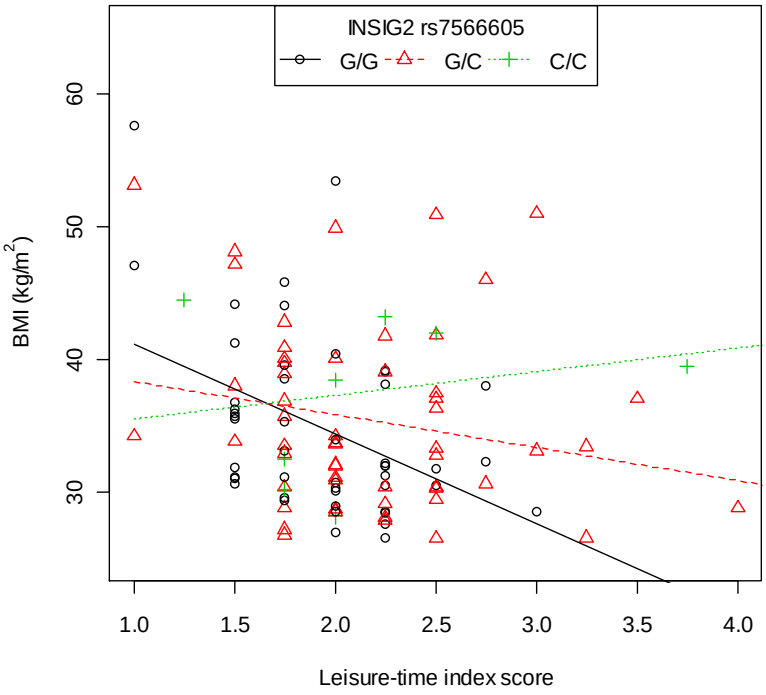

Figure 4.6: Plot of BMI against the leisure-time index score of the Baecke physical activity questionnaire. Symbols represent individual observed values and regression lines show the expected relationships for each genotype of the *INSIG2* rs7566605 polymorphism (minor allele = C).

Dietary intake of indicator food groups

The mean±SD frequency of daily intakes of high fat foods (including take-away food), energy-dense snacks, take-away foods (as an independent fourth group) and energy-dense drinks are presented in Table 4.9. A one-unit increase in the daily intake of energy-dense drinks was significantly associated with a 1.70 kg/m^2 increase in BMI. Similar significant associations were found between a one-unit increase in the intake of high fat foods, energy-dense snacks and take-away food and a 0.40, 1.61 or 1.96 kg/m^2 increase in BMI respectively (Table 4.9).

The significant differences in mean intake of indicator food groups between genotype groups are presented in Table 4.10. Subjects with the wild-type CC homozygous genotype of the *GNB3* C825T polymorphism had higher intakes of energy-dense drinks and high-fat foods. Subjects with the homozygous mutant GG genotype of the *FTO* rs17817449 polymorphism had higher intake of high-fat foods. The mutant AA homozygotes of the *FABP2* Ala54Thr polymorphism and the wild-type TT homozygotes of the *FTO* rs1421085 polymorphism had higher intakes of take-away foods (Table 4.10).

The rate of change in BMI in response to a change in the intake of energy-dense snacks differed significantly between the genotype groups of the *GNB3* C825T polymorphism (Table 4.9). The most pronounced increase in BMI in response to an increase in the intake of energy-dense snacks was observed for the wild-type CC homozygotes (Figure 4.7).

Table 4.9: Mean±SD intake/day of indicator food groups, the main effect size (the average change in BMI in response to a one-unit unit increase in intake of energy-dense food groups) and corresponding p-values, as well as the interaction effect size and corresponding p-values of the interaction between energy-dense food groups and polymorphisms on BMI.

Energy-dense food groups	Frequency of intake per day Mean±SD	Effect of energy-dense food groups on BMI		Interaction between energy-dense food group × polymorphism on BMI			
		Main effect size	p-value*	Genes and Polymorphisms	Genotype p-value†	Minor allele Interaction effect size	Additive p-value§
Energy-dense drinks	1.0±1.3	1.70	**0.0001**	*FABP2* Ala54Thr	0.9774	-0.15	0.7910
				INSIG2 rs7566605	0.2018	-1.53	0.0788
				FTO rs1421085	0.3551	0.06	0.9373
				FTO rs17817449	0.5730	-0.56	0.3484
				ADRB3 Trp64Arg	0.8581	1.29	0.8581
				ADRB2 Arg16Gly	0.2529	1.25	0.1605
				GNB3 C825T	0.9389	0.08	0.9327
High fat foods	5.3±4.0	0.40	**0.0071**	*FABP2* Ala54Thr	0.7181	-0.01	0.9704
				INSIG2 rs7566605	0.7821	-0.08	0.8123
				FTO rs1421085	0.7006	0.27	0.3109
				FTO rs17817449	0.2983	0.08	0.7103
				ADRB3 Trp64Arg	0.7701	0.47	0.7701
				ADRB2 Arg16Gly	0.1406	0.47	0.1133
				GNB3 C825T	0.5321	0.06	0.8295
Energy-dense snacks	1.4±1.4	1.61	**0.0001**	*FABP2* Ala54Thr	0.3410	-0.06	0.9082
				INSIG2 rs7566605	0.4686	-1.05	0.1930
				FTO rs1421085	0.3802	-0.09	0.8831
				FTO rs17817449	0.1379	-0.71	0.1717
				ADRB3 Trp64Arg	0.8973	0.63	0.8973
				ADRB2 Arg16Gly	0.7699	-0.56	0.5024
				GNB3 C825T	0.0950	-1.25	**0.0312**
Take-away foods	0.6±0.8	1.96	**0.0069**	*FABP2* Ala54Thr	0.3917	-1.18	0.3660
				INSIG2 rs7566605	0.4133	-1.37	0.3631
				FTO rs1421085	0.6505	1.96	0.2809
				FTO rs17817449	0.1431	-2.27	0.1026
				ADRB3 Trp64Arg	0.6080	1.65	0.6080
				ADRB2 Arg16Gly	0.8654	-0.52	0.7208
				GNB3 C825T	0.3607	-0.20	0.8878

All statistical models were adjusted for gender

*Linear model of main effects of daily intake of energy-dense food groups on BMI.

†Linear model of the interaction between each energy-dense food group and genotype groups (categorised as wild-type, heterozygous and mutant genotypes) on BMI.

§ Linear model of the interaction between each energy-dense food group and the additive allelic variable (numerically coded as 0, 1 and 2 minor alleles) on BMI.

Table 4.10: Significant differences in intake of energy-dense food groups between genotype groups.

Energy-dense food groups	Polymorphisms	Genotypes	n*	Frequency of intake/day mean±SD	p-value[§]
Energy-dense drinks	*GNB3* C825T	CC	47	1.6±1.8[a]	**0.0003**
		CT	35	0.4±0.4[b]	
		TT	18	0.8±0.8[a,b]	
	GNB3 C825T	CC	47	1.6±1.8	**<0.0001**
		T-allele carriers	53	0.5±0.6	
Take-away foods	*FABP2* Ala54Thr	G-allele carriers	89	0.4±0.4	**0.0337**
		AA (mutant)	14	0.8±1.5	
	FTO rs1421085	TT (wild-type)	21	1.0±1.8	**0.0207**
		C-allele carriers	73	0.4±0.4	
High fat foods	*FTO* rs17817449	T-allele carriers	59	4.9±3.6	0.0502[†]
		GG	38	6.6±4.9	**0.0494**
	GNB3 C825T	CC	47	6.5±5.0	**0.0420**
		T-allele carriers	53	4.8±3.1	

*n vary due to missing values

[§] Gender-adjusted linear model, in the case of 3 categories the means with the same letter do not differ significantly using the Tukey post-hoc test

[†] Unadjusted linear model

Non-significant differences not included in table

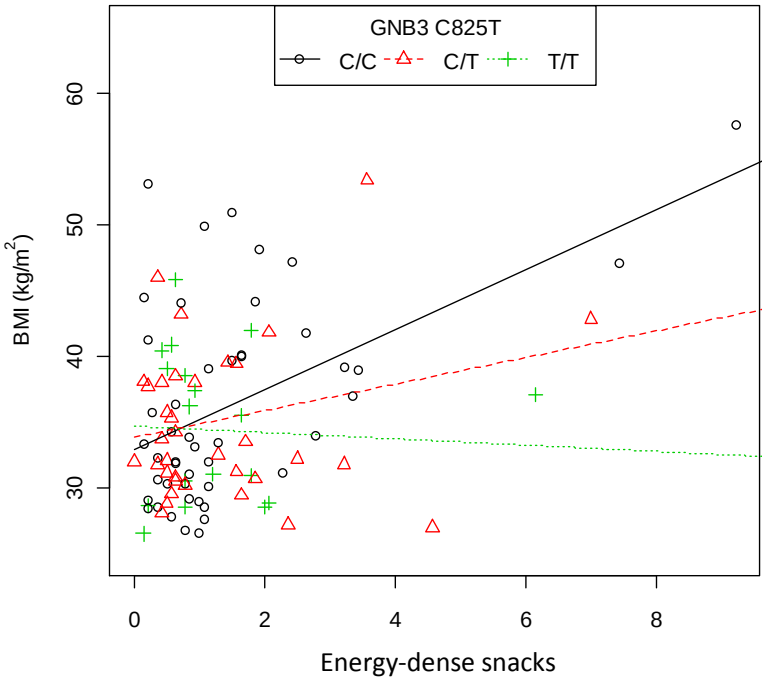

Figure 4.7: Plot of BMI against the intake of energy-dense snacks (high fat and carbohydrate extras). Symbols represent individual observed values and regression lines show the expected relationships for each genotype of the *GNB3* C825T polymorphism (minor allele = T).

Eating behaviour

The mean±SD scores derived from the TFEQ are presented in Table 4.11. A one-unit increase in the dietary restraint score was significantly associated with a 0.28 kg/m^2 decrease in BMI. A one-unit increase in the disinhibition, emotional disinhibition and habitual disinhibition scores was significantly associated with a 0.33, 1.46 and 1.25 kg/m^2 increase in BMI respectively (Table 4.11).

The significant differences in mean TFEQ scores between genotype groups are presented in Table 4.12. The mutant GG homozygotes of the *FTO* rs17817449 polymorphism had significantly higher rigid control and habitual disinhibition scores when using unadjusted linear models. However, after adjustment for gender these associations became non-significant. The mutant CC homozygotes of the *FTO* rs1421085 polymorphism had significantly higher hunger and specifically internal locus for hunger scores. The mutant C-allele carriers also had higher emotional disinhibition and flexible control scores that were dependent on gender, as these associations were non-significant in unadjusted models. The mutant CC homozygotes of the *INSIG2* rs7566605 polymorphism had a significantly lower attitude to self-regulation score of the dietary restraint subscale.

The rate of change in BMI in response to a change in rigid control and self regulation subscale scores differed significantly between the genotype groups of the *FTO* rs1421085 polymorphism (Table 4.11). The most pronounced increase in BMI in response to an increase in rigid control was observed for the mutant CC homozygotes (Figure 4.8). The most pronounced decrease in BMI in response to an increase in self regulation subscale scores was observed for the wild-type TT homozygotes (Figure 4.9). Furthermore, the most pronounced decrease in BMI in response to an increase in flexible control score was observed for the wild-type Arg16Arg homozygotes of the *ADRB2* Arg16Gly polymorphism (Figure 4.10). For the *INSIG2* rs7566605 polymorphism, the most pronounced increase in BMI in response to an increase in situational disinhibition score was observed for the mutant CC homozygotes (Figure 4.11).

Haplotype analysis revealed that the most pronounced increase in BMI in response to a decrease in the attitude to self-regulation subscale score (dietary restraint) was observed for the T-G haplotype compared to the C-T haplotype of the two FTO polymorphisms (effect size = 2.15, p=0.0393) results not presented in a Table). No significant differences were found between the other haplotype combinations.

Table 4.11: Mean±SD score of each scale/subscale of the TFEQ, the main effect size (the average change in BMI in response to a one-unit increase in TFEQ scores) and corresponding p-values, as well as the interaction effect size and corresponding p-values of the interaction between TFEQ scores and polymorphisms on BMI.

TFEQ scales and subscales	Score	Effect of TFEQ on BMI		Interaction between score × polymorphisms on BMI			
				Polymorphism	Genotype p-value†	Minor allele Interaction effect size	Additive p-value§
	Mean±SD	Main effect size	p-value*				
Factor I: Restraint	7.9±4.1	-0.28	**0.0439**	*FABP2* Ala54Thr	0.8882	0.14	0.5183
				INSIG2 rs7566605	0.1138	0.19	0.4175
				FTO rs1421085	0.3531	0.37	0.1612
				FTO rs17817449	0.2386	0.30	0.1743
				ADRB3 Trp64Arg	0.6936	0.22	0.6936
				ADRB2 Arg16Gly	0.6670	-0.20	0.3603
				GNB3 C825T	0.3691	0.23	0.2734
Restraint: Rigid control	2.4±1.5	-0.46	0.2364	*FABP2* Ala54Thr	0.8690	0.15	0.7995
				INSIG2 rs7566605	0.1374	0.39	0.5943
				FTO rs1421085	0.1334	1.67	**0.0290**
				FTO rs17817449	0.7153	0.01	0.9847
				ADRB3 Trp64Arg	0.7090	0.56	0.7090
				ADRB2 Arg16Gly	0.9357	0.20	0.7536
				GNB3 C825T	0.7205	0.28	0.6310
Restraint: Flexible control	2.3±1.6	-0.46	0.1944	*FABP2* Ala54Thr	0.7711	0.16	0.7859
				INSIG2 rs7566605	0.2866	0.71	0.2185
				FTO rs1421085	0.9609	-0.08	0.9002
				FTO rs17817449	0.4023	0.66	0.2117
				ADRB3 Trp64Arg	0.8624	0.31	0.8624
				ADRB2 Arg16Gly	**0.0438**	-1.50	**0.0161**
				GNB3 C825T	0.0666	1.08	0.0610
Restraint Strategic dieting	1.0±1.1	-0.96	0.0707	*FABP2* Ala54Thr	0.4489	0.90	0.3167
				INSIG2 rs7566605	0.5852	0.55	0.5537
				FTO rs1421085	0.5599	0.35	0.7079
				FTO rs17817449	0.1782	1.33	0.0744
				ADRB3 Trp64Arg	0.8722	0.35	0.8722
				ADRB2 Arg16Gly	0.6441	-0.64	0.4722
				GNB3 C825T	0.5649	0.87	0.2751
Restraint: Self-regulation	2.1±1.2	-0.60	0.2109	*FABP2* Ala54Thr	0.9231	0.45	0.5521
				INSIG2 rs7566605	0.6286	0.59	0.4740
				FTO rs1421085	**0.0362**	2.29	**0.0095**
				FTO rs17817449	0.1374	1.09	0.1491
				ADRB3 Trp64Arg	0.8957	-0.33	0.8957
				ADRB2 Arg16Gly	0.7528	-0.02	0.9777
				GNB3 C825T	0.7204	0.55	0.4278
Restraint: Avoidance of fatty foods	2.0±1.4	-0.59	0.1554	*FABP2* Ala54Thr	0.7567	0.52	0.4289
				INSIG2 rs7566605	0.2314	0.17	0.8312
				FTO rs1421085	0.9734	0.20	0.7966
				FTO rs17817449	0.2409	0.48	0.4472
				ADRB3 Trp64Arg	0.5405	0.99	0.5405
				ADRB2 Arg16Gly	0.2643	-0.98	0.1428
				GNB3 C825T	0.0534	0.13	0.8341

Table 4.11: *(continued)*

TFEQ scales and subscales	Score	Effect of TFEQ on BMI		Interaction between score × polymorphisms on BMI			
				Polymorphism	Genotype p-value†	Minor allele Interaction effect size	Additive p-value§
	Mean±SD	Main effect size	p-value*				
Factor II Disinhibition	10.5±3.7	0.33	**0.0309**	*FABP2* Ala54Thr	0.8497	-0.03	0.9202
				INSIG2 rs7566605	0.4700	0.12	0.6599
				FTO rs1421085	0.6270	-0.23	0.3794
				FTO rs17817449	0.3942	0.24	0.3396
				ADRB3 Trp64Arg	0.9969	0.00	0.9969
				ADRB2 Arg16Gly	0.5776	0.26	0.3177
				GNB3 C825T	0.4428	0.18	0.4950
Emotional disinhibition	2.2±1.2	1.46	**0.0056**	*FABP2* Ala54Thr	0.7107	-0.49	0.5324
				INSIG2 rs7566605	0.4333	-0.51	0.5975
				FTO rs1421085	0.3429	-1.34	0.1298
				FTO rs17817449	0.9805	-0.07	0.9292
				ADRB3 Trp64Arg	0.6907	-0.95	0.6907
				ADRB2 Arg16Gly	0.9364	0.30	0.7199
				GNB3 C825T	0.3075	0.09	0.9022
Situational disinhibition	3.4±1.4	0.02	0.9556	*FABP2* Ala54Thr	0.5981	-0.19	0.7877
				INSIG2 rs7566605	0.0839	1.76	**0.0280**
				FTO rs1421085	0.7466	-0.20	0.7844
				FTO rs17817449	0.4629	0.48	0.4651
				ADRB3 Trp64Arg	0.5711	1.42	0.5711
				ADRB2 Arg16Gly	0.7210	0.21	0.7688
				GNB3 C825T	0.5123	0.72	0.3187
Habitual disinhibition	2.8±1.5	1.25	**0.0011**	*FABP2* Ala54Thr	0.9565	-0.15	0.7837
				INSIG2 rs7566605	0.8538	-0.18	0.7713
				FTO rs1421085	0.4815	-0.70	0.2524
				FTO rs17817449	0.6520	0.41	0.4466
				ADRB3 Trp64Arg	0.7422	-0.54	0.7422
				ADRB2 Arg16Gly	0.2037	1.11	0.0757
				GNB3 C825T	0.3701	0.05	0.9311
Factor III: Perceived Hunger	7.2±3.5	0.15	0.3593	*FABP2* Ala54Thr	0.2395	-0.42	0.1137
				INSIG2 rs7566605	0.1707	0.39	0.2018
				FTO rs1421085	0.1942	-0.15	0.5869
				FTO rs17817449	0.2168	-0.27	0.3071
				ADRB3 Trp64Arg	0.8467	0.16	0.8467
				ADRB2 Arg16Gly	0.3478	0.37	0.1746
				GNB3 C825T	0.9877	-0.01	0.9778
Hunger: Internal locus of control	2.9±1.9	0.10	0.7573	*FABP2* Ala54Thr	0.2537	-0.74	0.1338
				INSIG2 rs7566605	0.3523	0.14	0.8061
				FTO rs1421085	0.1865	-0.09	0.8756
				FTO rs17817449	0.2603	-0.45	0.3326
				ADRB3 Trp64Arg	0.9705	0.05	0.9705
				ADRB2 Arg16Gly	0.1984	0.95	0.0705
				GNB3 C825T	0.4284	0.18	0.7150

Table 4.11: *(continued)*

TFEQ scales and subscales	Score	Effect of TFEQ on BMI		Interaction between score × polymorphisms on BMI			
				Polymorphism	Genotype p-value[†]	Minor allele Interaction effect size	Additive p-value[§]
	Mean±SD	Main effect size	p-value*				
Hunger: External locus of control	3.2±1.7	0.29	0.4035	*FABP2* Ala54Thr	0.3590	-0.72	0.1834
				INSIG2 rs7566605	0.2272	0.96	0.1150
				FTO rs1421085	0.2481	-0.28	0.6354
				FTO rs17817449	0.5111	-0.23	0.6733
				ADRB3 Trp64Arg	0.6801	0.79	0.6801
				ADRB2 Arg16Gly	0.8055	0.35	0.5243
				GNB3 C825T	0.7217	-0.27	0.6292

TFEQ = three factor eating questionnaire

All statistical models were adjusted for gender

*Linear model of main effects of each TFEQ scale or subscale score on BMI.

[†]Linear model of the interaction between each TFEQ scale or subscale score and genotype groups (categorised as wild-type, heterozygous and mutant genotypes) on BMI.

[§]Linear model of the interaction between each TFEQ scale or subscale score and the additive allelic variable (numerically coded as 0, 1 and 2 minor alleles) on BMI.

Table 4.12: Significant differences in mean scores of the total and subscale scores of the TFEQ by genotype groups of selected polymorphisms.

Questionnaire	Gene	Genotypes	n*	Score of questionnaire mean±SD	p-value
Restraint scale: Rigid control	*FTO* rs17817449	T-allele carriers	60	2.1±1.6	**0.0341**[†]
		GG	40	2.7±1.2	0.0513[§]
Restraint scale: Flexible control	*FTO* rs1421085	TT	20	1.7±1.6	0.0669[†]
		C-allele carriers	77	2.5±1.7	**0.0392**[§]
Restraint scale: self-regulation	INSIG rs7566605	G-allele carriers	102	2.2±1.1	**0.0424**[§]
		CC	8	1.4±1.4	
Habitual disinhibition	*FTO* rs17817449	T-allele carriers	60	2.6±1.6	**0.0479**[†]
		GG	40	3.2±1.5	0.0771[§]
Emotional disinhibition	*FTO* rs1421085	TT	20	2.0±1.3	0.2160[†]
		C-allele carriers	77	2.4±1.1	**0.0281**[§]
Hunger	*FTO* rs1421085	TT	20	5.9±3.4[a]	0.0276[§]
		TC	54	7.3±3.0[a,b]	
		CC	23	8.8±3.9[b]	
	FTO rs1421085	TT	20	5.9±3.4	**0.0373**[§]
		C-allele carriers	77	7.8±3.4	
	FTO rs1421085	T-allele carrires	74	6.9±3.2	0.0316[§]
		CC	23	8.8±3.9	
Hunger: Internal locus of control	*FTO* rs1421085	TT	20	2.2±1.7[a]	**0.0135**[§]
		TC	54	2.8±1.8[b]	
		CC	23	3.9±2.0[c]	

BDI = Beck depression inventory, RSES = Rosenberg self-esteem scale

*n vary due to missing values

[†] Unadjusted linear model

[§] Gender-adjusted linear model, in the case of 3 categories the means with the same letter do not differ significantly using the Tukey post-hoc test

Non-significant differences not included in table

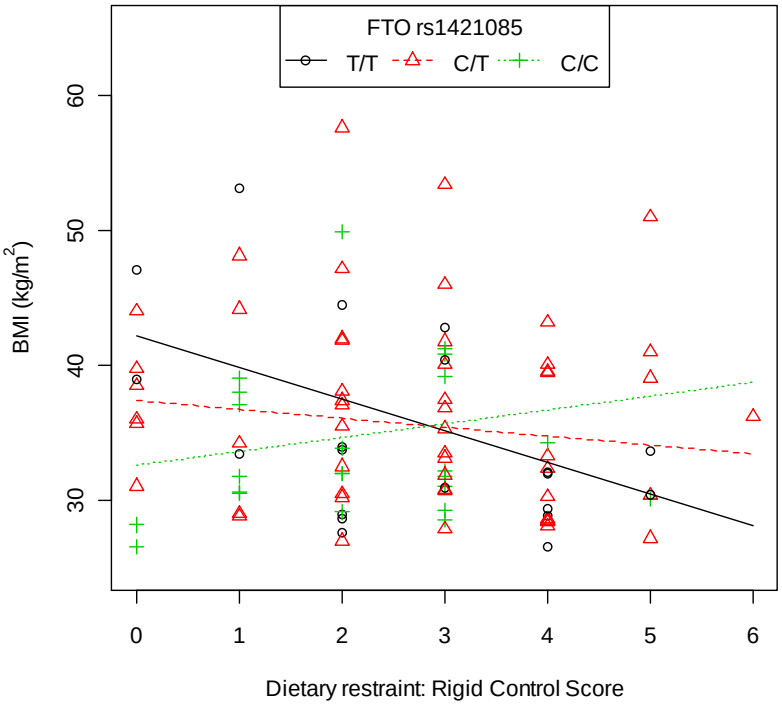

Figure 4.8: Plot of BMI against rigid control score (a subscale of the dietary restraint score of the TFEQ). Symbols represent individual observed values and regression lines show the expected relationships for each genotype of the *FTO* rs1421085 polymorphism (minor allele = T).

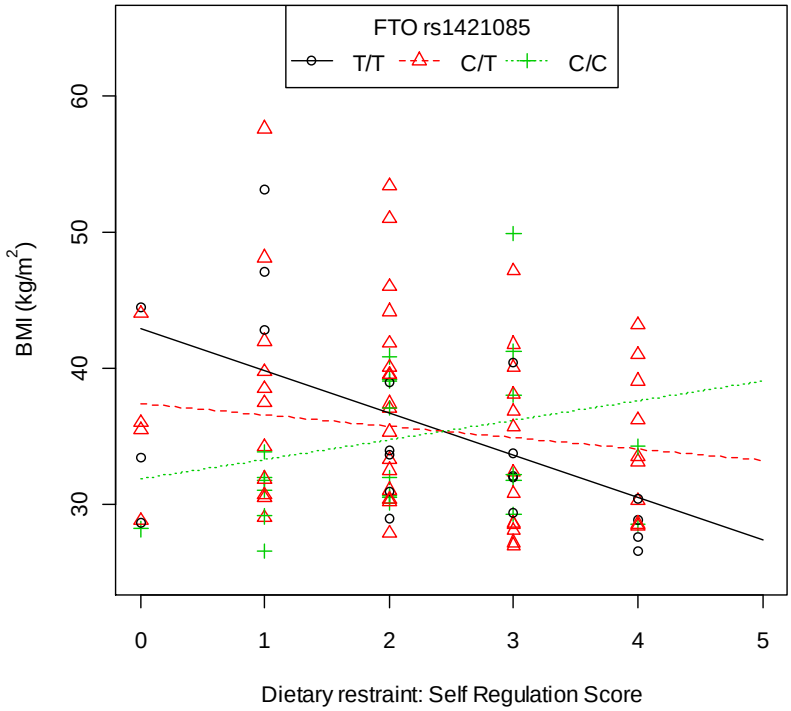

Figure 4.9: Plot of BMI against attitude to self-regulation score (a subscale of the dietary restraint score of the TFEQ). Symbols represent individual observed values and regression lines show the expected relationships for each genotype of the *FTO* rs1421085 polymorphism (minor allele = T).

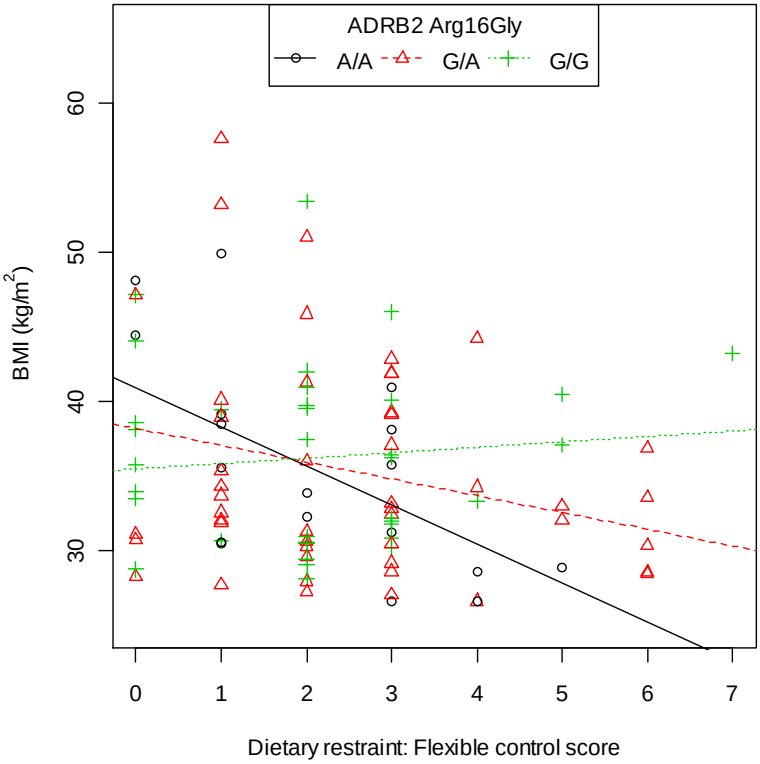

Figure 4.10: Plot of BMI against flexible control score (a subscale of the dietary restraint score of the TFEQ). Symbols represent individual observed values and regression lines show the expected relationships for each genotype of the *ADRB2* Arg16Gly polymorphism (minor allele = A).

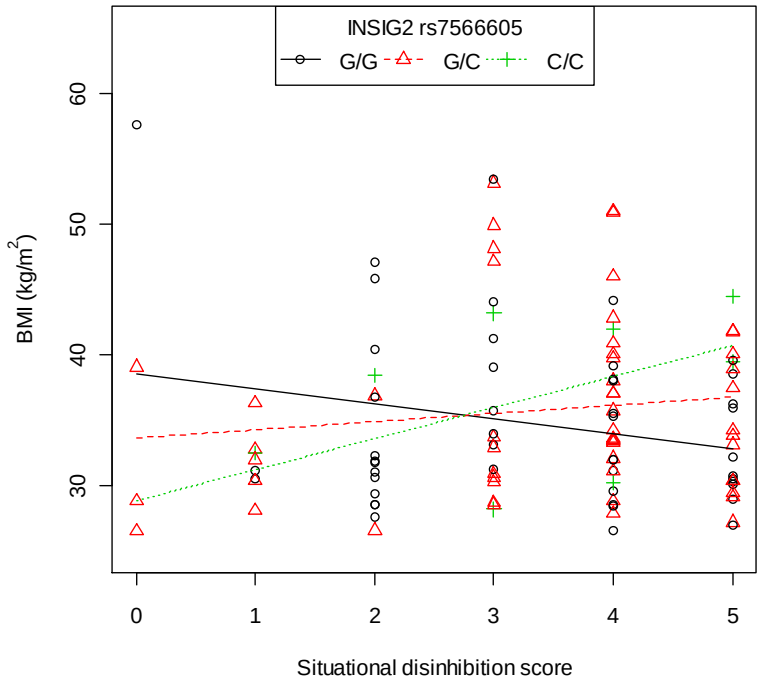

Figure 4.11: Plot of BMI against situational disinhibition score (a subscale of the disinhibition score of the TFEQ). Symbols represent individual observed values and regression lines show the expected relationships for each genotype of the *INSIG2* rs7566605 polymorphism (minor allele = C).

Psychological health

The mean±SD scores of the BDI, GHQ and RSES are presented in Table 4.13. A one-unit increase in the BDI score was associated with a 0.15 kg/m^2 increase in BMI. There were no associations between the scores of the GHQ or RSES and BMI (Table 4.13).

The significant differences in mean BDI and RSES scores between genotype groups are presented in Table 4.14. Significantly higher BDI scores were found in subjects with the wild-type CC homozygous genotype of the *GNB3* C825T polymorphism and in the mutant GG homozygotes of the *FTO* rs17817449 polymorphism (Table 4.14). The mutant TT homozygotes of the *GNB3* C825T polymorphism had a better self-esteem than wild-type allele carriers (Table 4.14). The mutant Thr54-allele carriers also had a better self-esteem than the wild-type Ala54Ala genotype, but this association was dependent on gender. No differences were observed between GHQ scores and the genotypes of the subjects (not presented in a Table).

The rate of change in BMI in response to a change in the GHQ score differed significantly between the genotype groups of the *FTO* rs1421085 polymorphism (Table 4.13). The most pronounced increase in BMI in response to an increase in GHQ score was observed for the wild-type TT homozygotes (Figure 4.12). No significant effect of the interaction between BDI or RSES scores and genotypes on BMI was found (Table 4.13).

Haplotype analysis revealed that the most pronounced increase in BMI in response to an increase in GHQ was observed for the T-G haplotype compared to the T-C haplotype of the two *FTO* polymorphisms (effect size = -0.655, p=0.469, results not presented in a Table). No significant differences were found between the other haplotype combinations.

Table 4.13: The main effect size (the average change in BMI in response to a one-unit unit increase in each psychological questionnaire score) and corresponding p-values, as well as the interaction effect size and corresponding p-values of the interaction between psychological questionnaire scores and polymorphisms on BMI.

Psychological questionnaire	Score	Effect of BDI, GHQ & RSES scores on BMI		Interaction between score and polymorphism on BMI			
				Polymorphism	Genotype p-value[†]	Minor alele Interaction effect size	Additive p-value[§]
	Mean±SD	Main effect size	p-value*				
BDI	14.0±9.9	0.15	**0.0094**	*FABP2* Ala54Thr	0.6750	0.10	0.3294
				INSIG2 rs7566605	0.9818	-0.01	0.9072
				FTO rs1421085	0.2868	-0.12	0.2710
				FTO rs17817449	0.9353	0.01	0.8992
				ADRB3 Trp64Arg	0.9193	-0.02	0.9193
				ADRB2 Arg16Gly	0.4002	-0.09	0.4284
				GNB3 C825T	0.2132	-0.04	0.6878
GHQ	15.6±5.8	0.06	0.5299	*FABP2* Ala54Thr	0.2121	0.23	0.1785
				INSIG2 rs7566605	0.4031	-0.18	0.2875
				FTO rs1421085	**0.0264**	-0.53	**0.0085**
				FTO rs17817449	0.5763	-0.15	0.3233
				ADRB3 Trp64Arg	0.2528	0.41	0.2528
				ADRB2 Arg16Gly	0.1631	-0.27	0.0882
				GNB3 C825T	0.9817	-0.03	0.8705
RSES	19.1±5.2	-0.21	0.0634	*FABP2* Ala54Thr	0.8811	0.09	0.6592
				INSIG2 rs7566605	0.4121	0.19	0.3354
				FTO rs1421085	0.2213	0.33	0.0969
				FTO rs17817449	0.4353	0.12	0.4221
				ADRB3 Trp64Arg	0.6105	-0.20	0.6105
				ADRB2 Arg16Gly	0.6507	-0.03	0.8848
				GNB3 C825T	**0.0377**	0.22	0.2119

BDI = Beck depression inventory, GHQ = General health questionnaire, RSES = Rosenberg self-esteem scale.

All statistical models were adjusted for gender

*Linear model of main effect of each psychological questionnaire score on BMI.

[†]Linear model of the interaction between each psychological questionnaire score and genotype groups (categorised as wild-type, heterozygous and mutant genotypes) on BMI.

[§]Linear model of the interaction between each psychological questionnaire score and the additive allelic variable (numerically coded as 0, 1 and 2 minor alleles) on BMI.

Table 4.14: Significant differences in mean scores of the BDI and RSES between genotype groups.

Psychological questionnaire	Gene	Genotypes	n*	Score of questionnaire mean±SD	p-value
BDI	*GNB3* C825T	CC	49	16.2±10.1	0.0570[†]
		CT	36	13.4±11.0	**0.0470**[§]
		TT	19	9.8±7.1	
	GNB3 C825T	C-allele carriers	85	15.0±10.5	**0.0363**[§]
		TT	19	9.8±7.1	
	GNB3 C825T	CC	49	16.2±10.1	**0.0354**[§]
		T-allele carriers	55	12.1±9.9	
	FTO rs17817449	TT	29	13.7±10.4[a,b]	**0.0454**[†]
		TG	40	11.7±8.5[a]	0.0736[§]
		GG	32	17.6±10.7[b]	
	FTO rs17817449	T-allele carriers	61	12.7±9.4	**0.0256**[§]
		GG	40	17.6±10.7	
RSES	*FABP2* Ala54Thr	GG (Ala54Ala)	57	17.8±5.0	0.0581[†]
		A-allele carriers	50	19.7±5.1	**0.0377**[§]
	GNB3 C825T	C-allele carriers	85	18.5±5.5	**0.0182**[§]
		TT	19	21.5±4.4	

BDI = Beck depression inventory, RSES = Rosenberg self-esteem scale

*n vary due to missing values

[†] Unadjusted linear model

[§] Gender-adjusted linear model, in the case of 3 categories the means with the same letter do not differ significantly using the Tukey post-hoc test

Non-significant differences not included in table

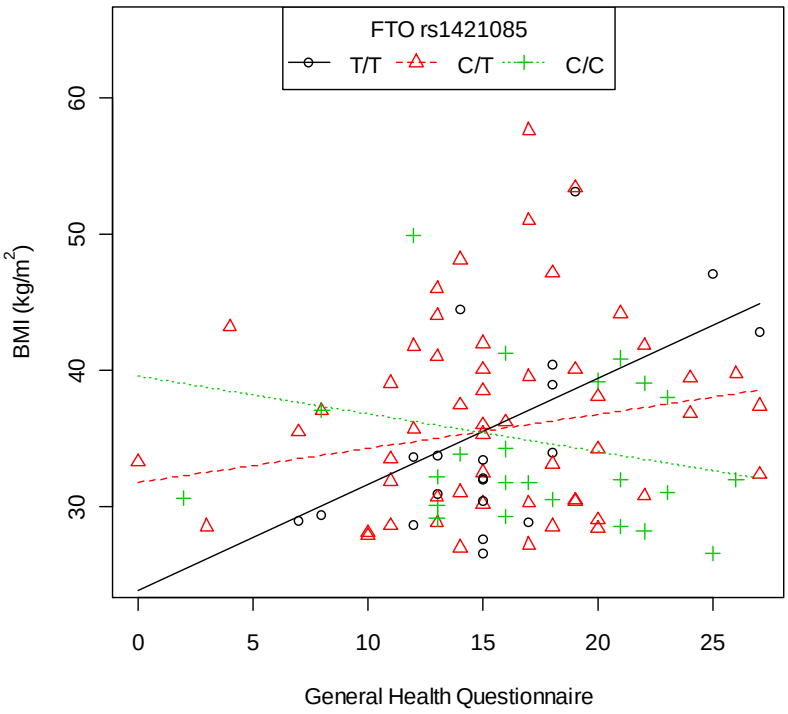

Figure 4.12: Plot of BMI against GHQ score. Symbols represent individual observed and regression lines show the expected relationships for each genotype of the *FTO* rs1421085 polymorphism (minor allele = T).

4.2 Discussion

This discussion firstly sets out to explore physical health of the study sample as reflected by the prevalence of the MetS and each MetS trait as well as the association thereof with BMI in overweight/obese Caucasians. Secondly, the associations between BMI and perceived weight history (as a proxy for actual weight status) as well as selected lifestyle (physical activity, dietary intake, eating behaviour) and psychological health variables are explored. The main focus of this chapter, namely the associations between genotype and BMI, health and lifestyle indicators as well as the effect of the interaction between genotype and these indicators on BMI, are explored in depth.

Metabolic syndrome and associations with BMI

Of the five MetS traits investigated in this study it was evident that abdominal obesity (a large waist circumference) was the most prevalent trait diagnosed in 93% of subjects according to the NCEP ATP III cut-offs. Compared to other studies using the same diagnostic criteria this prevalence was higher than those

reported for Caucasian South African samples, namely 40% in corporate executives that included individuals from all BMI categories (Ker *et al*. 2007) and 80% for women and 55% for men with type 2 diabetes (Kalk & Joffe 2008). It was also higher than the prevalence reported for Caucasians from Venezuela namely 34% for men and 53% for women (Florez *et al*. 2005) as well as the 48% reported for overweight/obese Italians (Bo *et al*. 2007) and 35% in Southern European populations (Bellia *et al*. 2009).

The expected positive association between BMI and waist circumference (Mikkola *et al*. 2007, Esteghamati *et al*. 2008) was found in this sample. Subjects who were classified with abdominal obesity according the ATP III criteria had a higher mean BMI than those who fell in the normal range for this trait. The association between waist circumference and BMI in our study is also reflected in the finding that a modelled 1 kg/m^2 increase in BMI is associated with a 2.44 cm increase in waist circumference. These associations between BMI and waist circumference explain the lower prevalence for abdominal obesity found in the abovementioned population samples when compared to our sample. It also explains the higher mean waist circumferences for men (118 cm) and women (107 cm) found in our overweight/obese sample compared to the waist circumferences reported for the Caucasian South African population (men = 91 cm; women = 80 cm) in the 2003 South African Demographic and Health Survey (SADHS) (DoH 2007). The fact that the mean waist circumferences in our sample were well above the cut-offs of 88 cm for women and 102 cm for men (ATP III criteria) and that the majority of the sample were diagnosed with abdominal obesity is concerning, as an increase in waist circumference is known to be associated with increased visceral fat that has also been related to hypertension, dyslipidemia, hyperglycemia and insulin resistance (Stolar 2007).

Dyslipidemia reflected by low HDL levels was the second most prevalent MetS trait in our sample (68%). This prevalence was higher than the prevalence reported for other Caucasian South African samples namely 38% for corporate executives (Ker *et al*. 2007) and 65% for women or 38% for men with type 2 diabetes (Kalk & Joffe 2008). In other Caucasian samples the prevalence was 8% for overweight/obese Italians (Bo *et al*. 2007), 57% (Bellia *et al*. 2009) and 62% (Florez *et al*. 2005) for population samples.

Subjects who were classified with a low HDL according the ATP III criteria had a higher mean BMI than those who fell in the normal range for this trait. The significant association that was found between a higher BMI and lower HDL levels was also evident in the fact that a modelled 1 kg/m^2 increase in BMI was associated with a 0.18 mmol/L decrease in HDL. This can also be interpreted as indicating that weight loss will help overweight/obese subjects to increase their HDL levels. This is in line with findings that indicate a positive effect of weight loss on increasing HDL levels (Williams *et al*. 1994, Abete *et al*. 2010, Asztalos *et al*. 2010). However, according to a review by Abete *et al*. (2010) evidence regarding the effect of weight loss on HDL levels is equivocal, with many studies indicating decreases in HDL levels, while some indicate no

changes or an increase in HDL levels. Differences in the macronutrient composition of the weight loss diets used in the studies review by Abete *et al*. (2010) may explain the contradictory results to some extent. Conventional weight loss diets (fat = 30%, protein = 15% and carbohydrate = 55% of TE) have mostly been found to decrease HDL levels; while low fat (20-30% of TE), high protein (20-30% of TE) diets have mostly been found to increase HDL levels. Furthermore, weight loss diets emphasizing a higher MUFA or omega-3 PUFA content have also been found to increase HDL levels (Abete *et al*. 2010).

Hypertension was the third most prevalent MetS trait in this sample of overweight/obese Caucasians. The hypertension prevalence of 42% was higher than the 29% reported for the corporate executives (Ker *et al*. 2007), but lower than the 72% reported for individuals with type 2 diabetes (Kalk & Joffe 2008). It was in line with the 37% (Florez *et al*. 2005) and 43% (Bellia *et al*. 2009) reported for other Caucasian population samples but lower than the 71% reported for overweight/obese Italians (Bo *et al*. 2007). The fact that our sample consisted of overweight/obese subjects can explain the higher prevalence of hypertension when compared to the corporate executive sample that included individuals from all BMI categories (Ker *et al*. 2007), as it is known that a positive association between BMI and BP exists (Misra & Khurana 2008). Similarly, when compared to the 2003 SADHS our hypertension prevalence was also higher than the prevalence reported for Caucasian women (15%) and men (36%) (DoH 2007). It must be borne in mind that the hypertension cut-off values set according to the ATP III criteria (135/85) differ from the cut-offs (140/90) used in the SADHS. Nonetheless our mean SBP/DBP of 132/88 was also higher than the 131/80 or 120/75 reported for South African Caucasian men and women respectively (DoH 2007).

Subjects who were classified with hypertension according to the ATP III criteria had a higher mean BMI than those who fell in the normal range for this trait. Furthermore, the significant association that was found between a higher BMI and a higher systolic or diastolic blood pressure in this sample of overweight/obese subjects was also reflected in the finding that a modelled 1 kg/m^2 increase in BMI was associated with a 4.55 mmHg increase in SBP and 5.56 mmHg increase in DBP. It is known that an increase in weight is a major risk factor for increased BP (Romero *et al*. 2007, Da Silva *et al*. 2009) and that small weight losses may decrease BP and the risk to develop MetS (Neter *et al*. 2008, Aucott *et al*. 2009). The association found between BMI and BP can also be interpreted inversely namely that a 1 kg/m^2 decrease in BMI may result in a 4.55 mmHg or 5.56 mmHg decrease in SBP and DBP respectively. These findings are in line with results from review articles and meta-analyses that typically suggest that a 5 kg weight loss (which is typically equal to a 1-2 kg/m^2 BMI reduction depending on initial weight and height), results in an average of 5.6 mmHg decrease in SBP (Aucott *et al*. 2009) whereas a 1 kg weight loss results in a 1 mmHg reduction in both DBP and SBP (Neter *et al*. 2008). Such changes in BP are beneficial as a Cochrane article of cohort studies indicated that the estimated effect of a 5 mmHg reduction in DBP is a 21% reduction in coronary heart disease and a 34% reduction in stroke (Brunner *et al*. 2007).

Abnormal triglyceride and glucose levels were the least prevalent MetS traits in this sample. Elevated triglycerides were diagnosed in 19% of the sample, which is lower than the prevalence of 81% reported in corporate executives (Ker *et al*. 2007) or 67% for women and 65% for men with type 2 diabetes (Kalk & Joffe 2008). The prevalence in other Caucasian samples was higher ranging from 30% (Bellia *et al*. 2009), 31% (Florez *et al*. 2005) to 36% (Bo *et al*. 2007). The prevalence of abnormal glucose levels (7%) in our sample was lower than the 12% reported for corporate executives (Ker *et al*. 2007), in line with the 8% reported for Caucasians from Venezuela (Florez *et al*. 2005) but lower than the 18% for overweight/obese Italians (Bo *et al*. 2007) or 20% for Southern European Caucasians (Bellia *et al*. 2009).

No associations were found between BMI and triglyceride or glucose levels in this study. This is in contrast with the general expected association between a higher BMI or obesity status and increased triglyceride or glucose levels (Stipanuk 2006). However, it must be borne in mind that an association between BMI and all MetS traits is not reported in all studies (Dixon & O'Brien 2001, Lemieux *et al*. 2006, Mikkola *et al*. 2007). This, together with the differences that were observed in the comparisons of the prevalence of MetS traits between our sample and the other Caucasian samples, may indicate that specific MetS traits as well as the association thereof with BMI may be population specific. It also needs to be considered that the variation in triglyceride and glucose levels in our overweight/obese sample was small, which may explain the lack of association with BMI. The fact that individuals with type 2 diabetes were excluded from the study may explain the low variation found in glucose values to some extent. The review by Abete *et al*. (2010) on the effect of weight loss programmes on MetS outcomes supports the fact that the expected association between glucose and triglycerides and BMI are not consistently found. Although the weight loss achieved by overweight/obese participants in the reviewed weight loss interventions mostly resulted in reductions in triglyceride and glucose levels, a number of studies also reported no effect on triglyceride or glucose levels, while some reported an increase in triglyceride levels. These differences may have resulted from specific dietary components or the differences in macronutrient composition of the diets (Abete *et al*. 2010).

MetS as such was diagnosed in almost half (47%) of the subjects using the NCEP ATP III criteria. This is the first South African research that specifically focussed on MetS in Caucasian overweight/obese adults. When considering other studies using the same diagnostic criteria, but including Caucasian South Africans from all BMI categories, a lower prevalence of 31% was found in corporate executives (Ker *et al*. 2007) and 26% in urban women (Greyling *et al*. 2007). These other South African figures are in line with the prevalence reported for other Caucasian samples namely 30% in Southern European populations (Bellia *et al*. 2009) and 37% in Caucasians from Venezuela (Florez *et al*. 2005). However, a higher MetS prevalence was reported for Caucasian South African men (74%) and women (84%) with type 2 diabetes (Kalk & Joffe 2008). Bo *et al*. (2007) reported a higher MetS prevalence in overweight/obese Caucasian Italians (27%)

compared to their normal weight counterparts (5%) (Bo *et al*. 2007). Our prevalence is in line with obese Caucasian American men (60%) and women (50%) (Park *et al*. 2003)

Although normal weight individuals are not exempt from developing MetS, a higher prevalence is generally expected in obese individuals (Park *et al*. 2003, Alberti *et al*. 2010). This may explain the higher prevalence found in our overweight/obese sample compared to the cross-sectional samples consisting of individuals with lower mean BMIs. When considering the association between BMI and diagnoses of MetS in our sample, it was evident that overweight/obese subjects who were diagnosed with MetS had a higher BMI, with each modelled 2.4 kg/m^2 increase in BMI being significantly associated with the diagnosis of an additional MetS trait.

In summary, it can be said that the physical health of the Caucasian overweight/obese subjects in this research was compromised as almost half were diagnosed with MetS mainly because of a large waist circumference, low HDL levels and hypertension. The results also indicate that those with a higher BMI may have an increased MetS risk. This stems from the fact that a higher BMI was associated with a larger waist circumference, a lower HDL and a higher systolic and diastolic blood pressure in this sample. Furthermore, subjects who were classified with having abdominal obesity, dyslipidemia (low HDL) and hypertension according to the ATP III cut-offs had a higher mean BMI than subjects who fell in the normal ranges for these traits. From the modelled associations it can be concluded that weight loss may help overweight/obese subjects to achieve a smaller waist circumference, higher HDL levels, a lower BP and having less MetS traits that met the diagnostic cut-offs.

Association between BMI and perceived weight history, lifestyle and psychological health variables

When exploring the weight history of a sample of adults, it is often not possible to access actual weight records and researchers resort to proxy indicators. In this study, perceived weight during childhood, adolescence and young adulthood were used as a proxy for the actual weight history of subjects. Retrospective follow-up of cohorts have indicated that women's perceived weight status during childhood, adolescence and adulthood accurately reflects their actual measured BMIs during those life-cycle stages (Munoz *et al*. 1996, Koprowski *et al*. 2001, Must *et al*. 2002). It has also been indicated that perceived weight accurately reflects actual weight in overweight adults, although normal weight women may be more likely to perceive themselves as overweight (Paeratakul *et al*. 2002; Linder *et al*. 2010). Paeratakul *et al*. (2002) reported that the best agreement between actual and perceived weight was found for overweight Caucasian women and individuals with a higher educational status and income level. Because the sample in this study included only overweight/obese adults who were mostly women with a tertiary level educational status and moderate to high income level, the assumption can be made that perceived weight history reflects actual weight status during each life-cycle stage.

The prevalence of subjects perceiving themselves as overweight/obese increased from childhood to adolescence and then to young adulthood. It was evident that a third of the subjects perceived their weight as overweight/obese during childhood, which increased to half of the sample during adolescence and to two-thirds during young adulthood. The subjects who thought that they were overweight/obese during a particular life-cycle phase also had a higher actual mean BMI (which was classified in the obese class II category of ≥35 kg/m^2) at the time of this study. Furthermore, subjects who thought that they were already obese in their young adulthood years, had a morbidly obese BMI of ≥40 kg/m^2 at the time of the study. If it is argued that perceived weight during a life-cycle phase is a proxy for actual weight during that life cycle phase, these results could firstly indicate that individuals between the ages of 25 to 40 years with a BMI≥35 kg/m^2 are very likely to have been overweight since childhood. It also supports the notion that obesity develops gradually over time (Williamson *et al*. 1990, Rothacker & Blackburn 2000, Rzehak & Heinrich 2006) and will manifest earlier in adult life depending on the age of onset of weight problems. These results are in line with cohort studies that found that childhood or adolescent weight or overweight status predicts adult weight or obesity status (Guo *et al*. 2002, Janssen *et al*. 2005, Must *et al*. 2005, Mamun *et al*. 2009). Adult overweight/ obesity status may therefore implicate life-long incorrect lifestyle choices such as an unhealthy diet and/or decreased physical activity levels that promote weight gain. However, it must also be borne in mind that some individuals may be normal weight during all the early life-cycle stages and only start gaining weight during young adulthood, thus only becoming overweight/obese during the investigated age group (25-40 years). This was reflected in the fact that a third of the subjects in our research indicated that they thought their weight was still normal when they were 20 to 25 years old. This possibility is supported by information provided by large prospective cohort studies, namely that unwanted weight gain commonly occurs between the ages of 18 to 34 years in both men and women (Braddon *et al*. 1986, Burke *et al*. 1996, Rothacker & Blackburn 2000, Williamson *et al*. 1990). Furthermore, it has also been shown that weight gain is particularly experienced after school when studying at a tertiary educational institution (Anderson *et al*. 2003, Graham & Jones 2002, Hodge *et al*. 1993).

Physical activity is one of the most important lifestyle behaviours contributing to effective weight management during all life-cycle phases. Physical activity does not only increase energy expenditure, but also contributes to the suppression of hunger, improvement in psychological well-being and the increased ability to oxidize fat instead of carbohydrates for a fuel (summarized by WHO 2000). In this sample of overweight/obese adults a lower BMI was associated with higher physical activity levels as measured by the sport index and leisure-time index scores of the Baecke physical activity questionnaire. The results indicate that a modelled one-point increase in the sport index or leisure-time index scores was respectively associated with a 2.01 kg/m^2 and 2.43 kg/m^2 decrease in BMI. In practical terms, a one-point increase in

the sport index score of a physically inactive subject could be brought about by practicing a low-level activity such as walking for 1-2 hours per week. Subjects who already walk 1-2 hours per week should increase this to 3-4 hours per week and additionally practice a medium level activity such as swimming, jogging, aerobics or cycling for at least one hour per week. Instead of increasing walking time they can also just practice the medium level activity for 3-4 hours per week to achieve a one-point increase in their sport index score (all recommendations were calculated using the equations for calculating the sport index score by Baecke *et al*. 1982). Similarly, to achieve an increase in the leisure-time activity score, subjects must watch less television and do more activities such as walking or cycling during leisure-time (Baecke *et al*. 1982). These proposed changes in terms of physical activity levels that need to be implemented to achieve a lower BMI are realistic and achievable. It is also in line with the current South African recommendations of 'accumulating 30 minutes or more of moderate-intensity physical activity on most, preferably all, days of the week' (Lambert *et al*. 2001). It is known that the benefits of physical activity are not limited to weight maintenance or weight loss but also involve improvement of many other health indicators. In a review by Ross and Janiszewski (2008) it was indicated that the benefits of physical activity to decrease cardiovascular disease risk may also be exerted independent of whether weight loss is achieved. It has been shown that physical activity has favourable effects on several risk factors for chronic diseases of lifestyle (CDLs) such as to decrease triglyceride levels, blood pressure, insulin resistance and to increase HDL levels (Blair & Morris 2009, Yung *et al*. 2009). The risk of developing MetS and other CDLs can thus be lowered by lifestyle changes including physical activity and a healthy dietary intake (Stolar 2007, Abete *et al*. 2010).

The focus of the dietary intake assessment in this study was on frequency of intake of energy-dense foods that may increase total energy intake and have been associated with weight gain and the development of obesity (Bray *et al*. 2004a, Rosmond *et al*. 2004, Black & Macinko 2008, Malik *et al*. 2006, Gibson 2008, Wolff & Dansinger 2008). The energy-dense indicator food groups investigated included high fat foods (including take-away foods), take-away foods as an independent group, energy-dense snacks, and energy-dense drinks. The total daily frequency of intake from these energy-dense food groups was found to be 7.7 times per day. The food based dietary guidelines (FBDG) for South African adults recommend that high fat foods such as full cream milk, red meat with fat, fried fish, sausages, take-away foods (hamburgers, chips, pies, etc.), margarine/butter, eggs prepared with fat, normal mayonnaise etc. should be eaten sparingly and substituted for leaner or low fat options on a daily basis in order to maintain weight and prevent the development of CDLs (Wolmarans & Oosthuizen 2001). However, with a mean frequency of intake of 5.3 times per day from the high fat foods indicator food group, it can be speculated that the overweight/obese subjects in this sample are not following these guidelines. It can also be speculated that this may probably have contributed to the development/maintenance of their current overweight/obese weight status. Although there are no specific South African FBDG for the intake of energy-dense snacks and drinks, the intake of foods from the latter two indicator food groups, which are typically high in fat and/or added

sugar/refined carbohydrates such as crisps, chocolate, sweets, cake etc. (energy-dense snacks) and carbonated soft drinks, energy drinks, juice etc. (energy-dense drinks), should be limited to avoid excessive energy intake. However, the subjects in this sample had a mean frequency of intake from these indicator food groups of 2.4 times per day, which does not reflect an occasional intake but rather a frequent daily intake.

It was indeed evident that in this sample of overweight/obese subjects, those with a higher BMI had a higher intake of each of the energy-dense indicator food groups. More specifically, a modelled increase in the frequency of intake of high fat foods, take-away foods, energy-dense snacks and drinks by one time per day was significantly associated with a 0.40, 1.96, 1.61 or 1.70 kg/m^2 increase in BMI respectively. From these main effect sizes it was evident that a more frequent intake of take-away foods was associated with the highest modelled increase in BMI. This is in line with research that has consistently show that a higher intake of take-away foods is associated with a higher BMI, greater long-term increases in weight and a higher risk of being obese (Duffey *et al*. 2007, Larson *et al*. 2009). This can be explained by the fact that the consumption of take-away foods has been associated with having higher total energy intakes (Bowman & Vinyard 2004, Schröder *et al*. 2007, Stender *et al*. 2007, Black & Macinko 2008, Rosenheck 2008).

A higher frequency of intake of these energy-dense indicator food groups may be reflected in/be the result of poorer eating behaviours. This possibility is supported by the fact that a higher BMI in our subjects was associated with having poorer eating behaviours, more specifically with lower dietary restraint, higher disinhibition, higher emotional disinhibition and higher habitual disinhibition scores. This is in line with previous work that showed that disinhibition scores are consistently associated with a higher weight, BMI or with weight gain over time (Bryant *et al*. 2008, Chaput *et al*. 2009, Rideout & Barr 2009, Savage *et al*. 2009). From the current research it was evident that a modelled one-unit increase in the disinhibition, emotional disinhibition and habitual disinhibition scores was significantly associated with a 0.33, 1.46 and 1.25 kg/m^2 increase in BMI respectively. It is known that individuals with a higher disinhibition score are more likely to overeat when food is very palatable, when in the company of someone who overeats, when at social occasions, when having an emotional or personal problem or when stressed (Stunkard & Messick 1985, Bryant *et al*. 2008). A review by Bryant *et al*. (2008) indicated that individuals with higher disinhibition scores prefer high fat foods, high-fat and high-salt foods, processed meats, sweet fruits and vegetables and sweet carbonated drinks and have higher intakes of sweets, cookies, ice cream, butter, coffee and alcohol. Our results also confirm previous reports that indicate that a lower restraint score is associated with a higher body weight (Hainer *et al*. 2006, Rideout & Barr 2009). It is also known that a decrease in dietary restraint over time is associated with weight gain (Savage *et al*. 2009), emphasizing the need for strategies that focus on improving dietary restraint as part of weight management interventions. Our results support this view as a modelled one-unit increase in the dietary restraint score was significantly

associated with a 0.28 kg/m^2 decrease in BMI. A higher restraint score is associated with being able to employ specific eating related strategies to consciously limit food intake in order to control body weight. These strategies include the avoidance of fattening foods, eating smaller portions, stopping/discontinuing eating before reaching satiation and consistently considering the energy content of food in order to control energy intake, as well as to buying low energy containing foods (Bryant *et al*. 2008). It has also been reported that the combined effect of dietary restraint and disinhibition influences body weight. The effect of disinhibition on body weight disappears when a high level of restraint eating is present (Hays *et al*. 2002, Savage *et al*. 2009). Therefore, individuals with the highest weight usually have the highest levels of disinhibition together with the lowest levels of restraint (Hays *et al*. 2002, Dykes *et al*. 2004), which is in line with our results.

In terms of psychological health we found that a higher BMI was significantly associated with having more depressive symptoms (BDI), with a modelled one-unit increase in BDI score being associated with a 0.15 kg/m^2 increase in BMI. Similar relationships between increased BMI and psychological health as well as several behavioural and psychological traits have been reported by others, including a lower body image, self-esteem, self-motivation and poor coping or problem-solving skills (Byrne 2002) as well as being depressed (Ahlberg *et al*. 2002, Davis 2009). Results from large cohort studies also indicate a higher prevalence of psychiatric disorders (including current depression, life-long depression and anxiety) in overweight or obese women or men compared to normal weight adults (Petry *et al*. 2008, Zhao *et al*. 2009). Although normal weight persons also suffer from depression, Murphy *et al*. (2009) compared obese and non-obese persons who had experienced a major depressive episode and found that the obese experienced longer episodes of depression, a larger number of episodes and they were five times more likely than the non-obese to overeat, which led to weight gain during a period of depression (Murphy *et al*. 2009). It is known that the intake of energy-dense foods such as sweet or fatty foods improves mood and alleviates effects of stress through opioid and dopamine neurotransmission or the serotonergic system. In a number of psychological conditions including depression, changes in these physiological pathways play a role in the development of obesity as there is a tendency to choose and overeat energy-dense foods when stressed or feeling depressed (reviewed by Gibson 2006). These studies together with our results emphasize the importance of improving the psychological health of subjects in weight management programmes in order for them to achieve success.

In summary, overweight/ obese subjects in the current sample who perceived themselves as overweight during their childhood, adolescence and young adulthood years had a higher mean BMI than those who perceived themselves as being normal weight during these life-cycle phases. Those who perceived themselves as obese during their young adulthood years were morbidly obese at the time of this study. The significant associations between BMI and lifestyle as well as psychological health variables provide

perspectives that could guide the development of weight management programmes aimed at overweight/obese Caucasian subjects. Based on the results of the modelled associations between BMI and specified variables it can be said that a reduction in BMI can be achieved by increasing sport and leisure-time physical activity; decreasing the intake of high-fat foods, energy-dense snacks, take-away foods and energy-dense drinks; improving dietary restraint and disinhibition and decreasing the presence of depressive symptoms.

Genotype and allele frequencies

Most of the genotype and allele frequencies of the polymorphisms tested were in line with those previously reported for other Caucasian samples, although some differences were evident. The wild-type Ala54Ala genotype frequency (46%) of the *FABP2* Ala54Thr polymorphism was within the range (44-55%) generally reported for obese and randomly selected Caucasian samples. However, the mutant Thr54Thr genotype frequency of 15% is double the reported range of 4-8%, while the mutant allele frequency was within the wide range of frequencies (25-47%) previously reported for Caucasian samples (Berthier *et al*. 2001, Damcott *et al*. 2003, Lara-Castro *et al*. 2005, Stan *et al*. 2005, Helwig *et al*. 2007, Morcillo *et al*. 2007, Tavridou *et al*. 2009).

For the *INSIG2* rs7566605 polymorphism the heterozygous genotype frequency was the highest at 48% shortly followed by the wild-type genotype frequency of 44%. This is in line with previously reported frequencies ranging between 41-48% for the heterozygous genotype and 43-52% for the wild-type genotype in other Caucasian samples. Similarly, our mutant CC genotype frequency of 8% and mutant C-allele frequency of 32% also fall within the respective ranges of 7-12% and 30-35% as previously reported for Caucasian samples (Loos *et al*. 2007, Lyon *et al*. 2007, Pollex *et al*. 2007, Rosskopf *et al*. 2007, Smith *et al*. 2007, Andreasen *et al*. 2008, Boes *et al*. 2008, Chu *et al*. 2008, Orkunoglu-Suer *et al*. 2008).

The genotype and allele frequencies of the *FTO* rs1421085 polymorphism were in line with those previously reported for obese Caucasian populations. In the latter obese samples, the mutant CC genotype frequency ranges between 20-28% (Dina *et al*. 2007b, Attaoua *et al*. 2008, Peeters *et al*. 2008, Price *et al*. 2008, Stutzmann *et al*. 2009) as was found in this study (25%), while a lower range of 11-18% has been reported in non-obese samples (Dina *et al*. 2007b, Attaoua *et al*. 2008, Do *et al*. 2008, Peeters *et al*. 2008, Price *et al*. 2008, Cauchi *et al*. 2009, Stutzmann *et al*. 2009). Although our wild-type genotype frequency of 21% falls just below the range (23-27%) reported in obese samples, both these are lower than the range (34-38%) found in non-obese samples. The frequency of the heterozygous genotype (55%) was the highest in our sample, which is in line with ranges of 44-55% previously reported by all studies independent of obesity status (Dina *et al*. 2007b, Attaoua *et al*. 2008, Do *et al*. 2008, Peeters *et al*. 2008, Price *et al*. 2008, Cauchi *et al*. 2009, Stutzmann *et al*. 2009). Furthermore, the high mutant C-allele frequencies (48-52%) that have

been reported for these obese samples (Dina *et al*. 2007b, Attaoua *et al*. 2008, Peeters *et al*. 2008, Price *et al*. 2008, Stutzmann *et al*. 2009) are similar to the frequency of 52% that was found in this study, which is higher than the 37-41% that has been reported for non-obese samples (Dina *et al*. 2007b, Attaoua *et al*. 2008, Do *et al*. 2008, Peeters *et al*. 2008, Price *et al*. 2008, Cauchi *et al*. 2009, Stutzmann *et al*. 2009).

A similar picture emerges when considering the *FTO* rs17817449 polymorphism. The mutant allele frequency of 55% in our subjects was just higher but still in line with the reported mutant allele frequency of 48-51% for obese samples (Dina *et al*. 2007b, Price *et al*. 2008), while a lower range of 36-40% was previously found in non-obese samples (Dina *et al*. 2007b, Do *et al*. 2008, Price *et al*. 2008). The frequency of the mutant genotype (40%) was the highest in our sample, while the wild-type and heterozygous genotype frequencies were similar at 29.5 and 30.5% respectively. The heterozygous genotype frequencies were found to be higher at 42-52% in other samples, while the frequency of the wild-type genotype at 23-27% in obese samples were more in line with ours than the 8-18% reported for non-obese samples (Dina *et al*. 2007b, Do *et al*. 2008, Price *et al*. 2008). Although these discrepancies in genotype and allele frequencies of the *FTO* rs17817449 polymorphism between this study and previous reports were found, it must be borne in mind that only three studies that included Caucasian samples could be traced, which narrowed the reported frequency ranges compared to the other polymorphisms for which 15 or more studies were available.

The wild-type Trp64Trp genotype frequency of the *ADRB3* Trp64Arg polymorphism was very high (90%) in our sample, while no subjects had the mutant Arg64Arg genotype. Similar patterns were reported for other Caucasian samples, with the wild-type genotype ranging between 82-90% and the mutant genotype between 0-1%. The frequency of the mutant Arg-allele (5%) was also in line with the 5-9% reported by others (Corella *et al*. 2001, Ramis *et al*. 2004, Kurokawa *et al*. 2008, Zafarmand *et al*. 2008).

For the *ADRB2* Arg16Gly polymorphism the frequency of the heterozygous genotype was the highest at 48%, followed by the mutant genotype at 34%. These genotype frequencies fall within the respective ranges of 37-53% and 32-46% reported for other Caucasian samples. The frequency of the mutant Gly16-allele (58%) was higher than that of the wild-type allele in our sample, but is similar to the range of 55-66% reported for Caucasian samples (Meirhaeghe *et al*. 2000, Oberkofler *et al*. 2000, Ellsworth *et al*. 2002, Dallongeville *et al*. 2003, Tafel *et al*. 2004, Mattevi *et al*. 2006).

The wild-type genotype and allele of the *GNB3* C825T polymorphism were the most frequent in our sample. The wild-type and heterozygous genotype frequencies of 49% and 34% fall within the respective ranges of 40-60% and 32-46% that was reported for other Caucasian samples. The mutant allele frequency (35%) is

similar to the range of 27-36% reported for other Caucasian populations (Brand *et al*. 2003, Wascher *et al*. 2003, Andersen *et al*. 2006, Danoviz *et al*. 2006, Grove *et al*. 2007, Renner *et al*. 2007, Casiglia *et al*. 2008).

In summary, with the exception of the *FTO* rs17817449 polymorphism, all other genotype and allele frequencies are similar to those reported for other Caucasian samples. The *FTO* rs17817449 genotype and allele frequencies best reflect the frequencies reported for the obese Caucasian subjects compared to the non-obese subjects in the two case-control and one population study cited earlier in this section. For the *FTO* rs1421085 polymorphism it was also evident that the frequencies found in this overweight/obese sample were in line with other obese Caucasian samples and are different from those reported for non-obese samples.

Genotype associations with BMI.

No differences in BMI between the genotype groups or additive allelic variable were found for any of the polymorphisms investigated in this sample of overweight/obese subjects. Our finding that the FABP2 Ala54Thr polymorphism is not associated with BMI is line with the results of the majority of association studies that included Caucasian populations and several large cross-sectional samples (Galluzzi *et al*. 2001, Damcott *et al*. 2003, Helwig *et al*. 2007). However, there were studies that found that the mutant Thr54-allele was associated with obesity (Morcillo *et al*. 2007, Travidou *et al*. 2009) and a higher BMI (Fisher *et al*. 2006) in Caucasian populations. The discrepancy in results may be attributed to the use of different study designs, genetic models in statistical analyses as well as subject heterogeneity for different diseases or metabolic conditions such as type 2 diabetes (Travidou *et al*. 2009).

The reported associations between the INSIG rs7566605 polymorphism and BMI or obesity have been conflicting. Although, the mutant CC genotype has been associated with an increased risk for obesity or with a higher BMI in case-control and cross-sectional studies, the majority of studies reported no association. A recent meta-analysis (Heid *et al*. 2009) that included 34 studies and 74345 subjects confirmed that there is no association when obese and normal weight controls were compared. However, these authors indicated that the INSIG rs7566605 polymorphism may play a role in extreme obesity as significant results were found when comparing normal weight controls to subjects with more extreme obesity (i.e. BMI>32.5; 35 or 40). In the latter subgroup analysis the CC genotype frequencies increased in each higher obese BMI category and consequently higher ORs and more significant p-values were reported for more obese subjects (Heid *et al*. 2009). The fact that we did not include a normal weight control group as well as our limited sample size may explain why we did not find a significant association with BMI.

Consistent evidence from small and large-scale studies shows that the mutant CC genotype or C-allele of the *FTO* rs1421085 polymorphism (Dina *et al*. 2007b, Attaoua *et al*. 2008, Do *et al*. 2008, Peeters *et al*. 2008,

Price *et al*. 2008, Cauchi *et al*. 2009, Stutzmann *et al*. 2009) and the mutant GG genotype or G-allele of the *FTO* rs17817449 polymorphism (Dina *et al*. 2007b, Do *et al*. 2008, Price *et al*. 2008) are associated with obesity and BMI in Caucasian populations with European ancestry. Study design limitations may explain why we could not repeat these reported associations as all the previous reports used case-control, cohort (BMIs ranged from underweight to obese) or family-based designs and none compared the association within an overweight/obese group only.

Our results are in line with those from the most recent meta-analyses in Caucasian populations that indicated no association between BMI and the ADRB3 Trp64Arg polymorphism (Kurokawa *et al*. 2008). Although it was indicated that the mutant Arg64-carriers had a higher BMI compared to the Trp64Trp homozygotes in the overall sample that included 97 studies and 44833 subjects, population heterogeneity effects were detected and subgroup analysis revealed that this association is only true for East Asian populations and not for Caucasians (Kurokawa *et al*. 2008).

In line with our findings, no association was reported between BMI and the ADRB2 Arg16Gly polymorphism in a study that included 7808 Caucasian subjects (Gjesing *et al*. 2007), a meta-analysis that included 13 studies with populations of different ethnicities (Gjesing *et al*. 2007) as well as other population-based (Meirhaeghe *et al*. 2000, Lima *et al*. 2007) and case-control studies (Large *et al*. 1997, Oberkofler *et al*. 2000, Terra *et al*. 2005) in Caucasians. Furthermore, the results of the positive association studies were equivocal with some linking the Gly16-allele or Gly16Gly genotype with a higher BMI (Ehrenborg *et al*. 2000) or with weight gain over a seven year period (Van Rossum *et al*. 2002) while others linked this genotype with a lower BMI (Meirhaeghe *et al*. 2001) or obesity prevalence (Ukkola *et al*. 2000). These discrepancies in the results may be explained by the different sample sizes included, study-designs used and the fact that some studies included only male subjects who were non-obese (Ehrenborg *et al*. 2000), while others included family based (Ukkola *et al*. 2000) or population-based (Meirhaeghe *et al*. 2001) samples.

Our results are in line with those from Rydén *et al*. (2002) who also reported no association between the GNB3 C825T polymorphism and BMI in a sample of obese Caucasians. Similar results were also reported for several population-based cross-sectional studies that included Caucasians (Benjafield *et al*. 2001, Rydén *et al*. 2002, Wascher *et al*. 2003, Martín *et al*. 2005, Terra *et al*. 2005, Andersen *et al*. 2006, Renner *et al*. 2007). In contrast, it has been found that the mutant TT homozygous genotype or T-allele carriers of the GNB3 C825T polymorphism are associated with a higher BMI (Siffert *et al*. 1999a, Siffert *et al*. 1999b, Benjafield *et al*. 2001, Poch *et al*. 2002, Stefan *et al*. 2004, Casiglia *et al*. 2008). A recent meta-analysis including 18903 subjects from 18 studies (of which 12 studies were performed in Caucasians, three in Asians, one in Africans and two in subjects with mixed ancestry) indicated a non-significant trend (p=0.053) towards a higher BMI in TT homozygotes (Souza *et al*. 2008).

Genotype associations with health and lifestyle indicators.

The significant associations that were found between genotype and perceived weight history, MetS traits, physical activity levels, dietary intake as well as eating behaviour and psychological health variables (secondary and exploratory hypotheses) are summarized in Table 4.15. The table also includes the significant modelled effect of the interaction between genotype and specified variables on BMI. In the sections following Table 4.15 the significant results reported in the table are discussed for each polymorphism. To provide context in the discussion reference is mostly made to relevant findings in other Caucasian populations. However, in some instances reference is also made to findings reported for populations from Asian or African ancestry. The ADRB3 Trp64Arg polymorphism is not included in the table or discussion as no associations were found between this polymorphism and the mentioned variables.

Table 4.15: Summary of the significant associations between specified variables and genotypes of each polymorphism as well as significant modelled effect of the interaction between genotype and specified variables on BMI.

Gene and polymorphism	**Genotype**	
	Wild-type	**Mutant**
***FABP2* Ala54Thr**	**Ala54Ala (GG)**	**Thr54Thr (AA)**
	*↑ BMI = ↑↑ triglycerides	
	(GG+GA): ↓ intake of take-away food	↑ intake of take-away food
	↓ self-esteem[§]	(AA+AG): ↑ self-esteem[§]
***INSIG2* rs7566605**	**GG**	**CC**
	(GG+GC): ↑ HDL	↓ HDL
	(GG+GC): ↓ number of MetS traits	↑ number of MetS traits
	↓ leisure-time activity score	(CC+CG): ↑ leisure-time activity score
	*↑ leisure-time activity = ↓↓ BMI	
	(GG+GC): ↑ self-regulation (restraint subscale)	↓ self-regulation (restraint subscale)
		*↑ situational disinhibition = ↑↑ BMI
***FTO* rs17817449**	**TT+TG**	**GG**
		*↓ BMI = ↑↑ HDL[§]
	↓ intake of high fat foods[§]	↑ intake of high fat foods[§]
	↓ Rigid control (restraint subscale)[†]	↑ Rigid control (restraint subscale)[†]
	↓ Habitual disinhibition[†]	↑ Habitual disinhibition[†]
	↓ BDI score	↑ BDI score
***FTO* rs1421085**	**TT**	**CC**
	↓ % perceived adolescent weight as normal	↑ % perceived adolescent weight as normal
		*↓ BMI = ↑↑ HDL
	↑ intake of take-away foods	(CC+CT): ↓ intake of take-away foods
	↓ Hunger score	(CC/ CC+CT): ↑ Hunger score
	↓ Internal locus for hunger	↑ Internal locus for hunger
	↓ emotional disinhibition[§]	(CC+CT): ↑ emotional disinhibition[§]
	↓ flexible control (restraint subscale)[§]	↑ flexible control (restraint subscale)[§]
	*↑ self-regulation = ↓↓ BMI	
		*↑ rigid control = ↑↑ BMI
	*↑ GHQ = ↑↑ BMI	
***FTO* haplotypes**	*T-T vs. T-G: ↑ sport index score = ↓ BMI	*T-G vs. T-C haplotype: ↑ GHQ = ↑ BMI and ↓ self-regulation = ↑ BMI
		C-G vs. T-T: *↓ BMI = ↑↑ HDL[§]

Table 4.15: *(continued)*

Gene and polymorphism	Genotype	
	Wild-type	**Mutant**
***ADRB2* Arg16Gly**	**Arg16Arg (AA)**	**Gly16Gly (GG)**
	↑ % Perceived childhood weight as overweight	(Gly16Gly+Arg16Gly): ↑ % Perceived childhood weight as normal
	*↑ flexible control = ↓↓ BMI	
***GNB3* C825T**	**CC**	**TT**
	CC: ↑ % Perceived childhood weight as normal CT: ↑ % Perceived childhood as overweight	↑ % Perceived childhood weight as normal
	(CC+CT): ↓ triglycerides[§]	↑ triglycerides[§]
	↓ glucose	↑ glucose
	(CC+CT): ↓ number of MetS traits	↑ number of MetS traits
	(CC/CT): ↓ % diagnosed with MetS	↑ % diagnosed with MetS
	*↑ leisure-time activity = ↓↓ BMI	
	*↑ high fat/carbohydrate snacks = ↑↑BMI	
	↑ high fat foods	(TT+TC): ↓ high fat foods
	↑ non-alcoholic drinks	(TT+TC): ↓ non-alcoholic drinks
	↑ BDI score	↓ BDI score
	(CC+CT): ↓ self-esteem	↑ self-esteem

*Reflects the modelled effect of the interaction between genotype and specified variables on BMI; all other entrees in the table indicate associations between genotype and specified variables only.
All results are significant for unadjusted and gender-adjusted analyses unless indicated as follows:
[§]Only significant for gender-adjusted results
[†]Only significant for unadjusted results

The FABP2 Ala54Thr polymorphism

The significant associations that were found for the *FABP2* Ala54Thr polymorphism involve TG levels, frequency of intake of take-away foods and self-esteem (Table 4.17).

The results show that a modelled increase in BMI of the wild-type Ala54Ala homozygotes was associated with an increase in TG levels. The opposite of this association can also be interpreted, namely that a modelled decrease in BMI is associated with a decrease in TG levels. It can therefore be suggested that weight loss may help to decrease TG levels in overweight/obese Ala54Ala homozygotes and consequently decrease their MetS risk profile. However, the same beneficial effect of weight loss on TG levels may not be observed for the mutant Thr54-allele carriers as no such association was found in these subjects. Although the exact physiological functions of *FABP2* are not yet fully elucidated (Helwig *et al*. 2007, Montoudis *et al*. 2008), it is known that the FABP2 proteins are expressed in the enterocytes of the GIT where they bind to dietary long chain saturated and unsaturated fatty acids and transport them from the brush border membrane to the ER, mitochondria, Golgi-apparatus and perinuclear area (Weiss *et al*. 2002, Cianflone *et al*. 2008, Karsenty *et al*. 2009). In the mitochondria FABP2 influences cholesterol biosynthesis as well as β-oxidation of fatty acids by regulating the expression of key enzymes. The Thr54 amino acid causes small structural changes in the portal region of FABP2 affecting its binding affinity to dietary fatty

acids (Zhang *et al*. 2003). The fact that the mutant Thr54 containing FABP2 protein has been found to have a greater binding affinity for dietary fat in the GIT (Baier *et al*. 1995) and has been associated with increased ApoBI and increased lipid synthesis, specifically with increased TG and phospholipid esterification and TG secretion (Baier *et al*. 1996, Levy *et al*. 2001), could explain our findings. It can be argued that a weight loss diet that results in weight loss and consequently decreased TG levels in Ala54Ala will not exert similar beneficial effects on TG levels of the Thr54-carriers, as they still absorb more dietary fat and secrete more TG than the Ala54Ala homozygotes. It can be hypothesized that the involvement of the FABP2 protein in gene expression of key β-oxidation and lipogenic enzymes may differentially change as a consequence of the mutant FABP2 protein, thus affecting blood lipid levels. The work by Marín *et al*. (2005) and Morcillo *et al*. (2007) further points to the possibility that the fatty acid composition of the diet may be more important to consider in weight loss than the total fat content. These authors have shown that Thr54-carriers experienced higher post-prandial lipid levels and decreased insulin sensitivity following diets high in SFAs, whereas diets low in total fat and SFAs or high in MUFAs were found to counter these effects (Marín *et al*. 2005). Morcillo *et al*. (2007) indicated that Thr54-carriers who regularly consumed sunflower oil (PUFA) had higher insulin resistance than those who regularly consumed olive oil (MUFA). The observation in rats that the Fabp2 protein has a greater affinity for linoleic acid (PUFA) than for oleic acid (MUFA) (Richieri *et al*. 1994) may elucidate these findings to some extent. When considering the intake of indicator food groups in our subjects, it was evident that the Thr54Thr homozygotes had a higher frequency of intake of take-away foods. This may compound the effect found on the TG levels of Thr54Thr homozygotes as described above as these foods are generally high in SFAs, PUFAs and TFAs. Although not reflected in the genotype differences for TG levels in our study or other studies that included Caucasian populations (Tahvanainen *et al*. 2000, Berthier *et al*. 2001, Damcott *et al*. 2003), a number of studies did report an association that points to the fact that Thr54Thr homozygotes have higher TG levels and a more unfavourable lipid profile compared to their wild-type counterparts (Agren *et al*. 1998, Carlsson *et al*. 2000, Georgopoulos *et al*. 2000, Galluzzi *et al*. 2001, Stan *et al*. 2005).

The currently known physiological functions of FABP2 do not provide a plausible explanation for the association found with self-esteem. It is thus possible that this result is a false positive and should thus be confirmed in further research.

In summary, novel findings for the *FABP2* Ala54Thr polymorphism that warrant further investigation include that 1) a modelled decrease in BMI of wild-type Ala54Ala subjects is associated with the most pronounced decrease in TG levels, while this may not be true for the mutant Thr54-carriers and 2) Thr54Thr homozygotes may have a higher frequency of intake of energy-dense foods that may compound the problem. These findings are supported by existing evidence indicating a higher fat absorption, specifically

SFA and PUFA absorption, in Thr54-allele carriers. The mutant Thr54Thr subjects also had a higher self-esteem, but this exploratory novel finding should be further investigated.

The INSIG rs7566605 polymorphism

The significant associations that were found for the *INSIG2* rs7566605 polymorphism involve HDL levels, the number of MetS traits diagnosed, leisure-time physical activity and certain eating behaviour variables.

Only one study (Pollex *et al*. 2007) could be traced that investigated the association between MetS and the *INSIG2* rs7566605 polymorphism. In line with our results, these authors reported no association between this polymorphism and MetS as diagnosed with the ATP NCEP III criteria in six Canadian populations with different ancestries (Pollex *et al*. 2007). Research on individual MetS traits in Caucasian populations showed no associations between this polymorphism and blood pressure, waist circumference, glucose, HDL and other blood lipids (Pollex *et al*. 2007, Andreasen *et al*. 2008, Boes *et al*. 2008, Chu *et al*. 2008, Peeters *et al*. 2009, Vimaleswaran *et al*. 2009). In contrast, our results show that subjects with the mutant CC genotype had significantly lower HDL levels compared to the G-allele carriers. The CC subjects also had a higher number of traits that met the criteria for the diagnosis of MetS. These associations might indicate that subjects with the mutant genotype have a higher risk to develop MetS. It can be argued that the associations with MetS traits seen in our CC subjects are supported by previous findings that show that Caucasians with the CC genotype have a higher BMI (Herbert *et al*. 2006, Lyon *et al* 2007, Rosskopf *et al*. 2007, Liu *et al*. 2008, Andreasen *et al*. 2008) and subcutaneous adipose tissue and fat volume (Franks *et al*. 2008, Orkunoglu-Suer *et al*. 2008). Furthermore, a recent meta-analysis (Heid *et al*. 2009) confirmed that the CC genotype frequencies are significantly higher in subjects with more extreme obesity (i.e. BMI>32.5; 35 or 40) when compared to normal weight controls. It is a known fact that a higher BMI and/or increased abdominal fat content increase MetS prevalence (Stolar 2007).

Insight in the physiological functioning of INSIG2 supports the possibility that the CC genotype's ability to block cholesterol and fatty acid synthesis as well as lipogenesis may be compromised, contributing to the development of MetS. It is known that the wild-type INSIG2 protein blocks the transcription of several genes of which the resulting protein products are required in the biosynthetic pathways of fatty acids, triglycerides, phospholipids, cholesterol, the NADPH cofactor and the LDL-receptor (Horton *et al*. 2002, DeBose-Boyd 2008). The wild-type INSIG2 protein therefore contributes to decreased synthesis of these lipids and thus lower plasma cholesterol and triglyceride levels (Yabe *et al*. 2002, Yang *et al*. 2002, Sun *et al*. 2005, Goldstein *et al*. 2006). It has also been indicated that *Insig2* expression in target cells increases following the consumption of a high fat diet, consequently blocking the activation of target lipogenic enzymes resulting in decreased preadipocyte differentiation and lipogenesis (Li *et al*. 2003, Takaishi *et al*. 2004). Gong *et al*. (2006) showed that a variant Insig2 protein investigated by their group lost the ability to

block gene transcription of enzymes required for biosynthetic pathways for cholesterol. However, it must be borne in mind that it is still unknown whether the *INSIG2* rs7566605 polymorphism results in the expression of such a variant INSIG2 protein. Furthermore, as the *INSIG2* rs7566605 polymorphism is located 10 kb upstream of the transcription start site of the *INSIG2* gene (Herbert *et al*. 2006), it is also still a question whether this variant itself or maybe another variant in strong LD leads to the expression of a variant INSIG2 protein. Hotta *et al*. (2008a) argue that the *INSIG2* rs7566605 polymorphism may affect the transcriptional activity of *INSIG2* and may therefore have an impact on its expression. Although many of the functional effects of this polymorphism still need to be elucidated, it is speculated that the mutant rs7566605 C-allele encodes a variant INSIG2 protein. This might explain why the mutant CC subjects in our study showed a stronger risk to develop MetS.

When considering physical activity levels, the C-allele carriers in our study were significantly more active during their leisure-time[7] than the GG homozygotes. Interestingly, the GG homozygotes experienced a pronounced decrease in BMI in response to a modelled increase in the leisure-time index score. This decrease in BMI in response to an increase in the leisure-time index score was less pronounced in the heterozygous subjects, while no association was found for the mutant CC homozygotes. These results firstly suggest that overweight/obese GG subjects may benefit more from interventions with a stronger focus on physical activity in order to achieve or maintain a lower weight; and secondly, they may find it easier to maintain weight or to lose weight as they respond to increased leisure-time physical activity.

The interaction between the INSIG2 rs7566605 polymorphism and physical activity has also been investigated by others as INSIG2 plays a role in adipocyte metabolism which in turn is affected by physical activity levels. Although these studies did not focus on leisure-time activity as such, but rather on total physical activity levels or specific exercise interventions, it was deemed valuable to consider the results of these reports in this discussion. It can be argued that the reduction in subcutaneous fat levels in the non-dominant arm of GG Caucasian men following a 12-week resistance exercise intervention focused on the mentioned arm (Orkunoglu-Suer *et al*. 2008) supports our finding that exercise has a more pronounced effect on decreasing fat levels and consequently weight in the GG genotype. This notion is further supported by the results of two weight loss interventions that included physical activity components and were targeted at obese Caucasian children aged between six to 16 years (Reinehr *et al*. 2008, Reinehr *et al*. 2009). In both studies, the G-allele carriers lost more weight after following the one-year intervention programme than CC homozygotes who gained weight during this period (Reinehr *et al*. 2008, Reinehr *et al*. 2009). However, the work by Franks *et al*. (2008) refutes this notion. These researchers found that CC homozygotes lost more weight compared to G-allele carriers after a one-year weight loss intervention that

[7] The leisure-time index of the Baecke physical activity questionnaire covers the amount of physical activity, other than sport, performed during leisure-time. The sport index of this questionnaire covers the duration and type of formal exercise or sport that a subject practice per week, however no associations with genotype were found.

included 150 min of physical activity per week (Franks *et al*. 2008). Furthermore, Andreasen *et al*. (2008) reported that the BMI of G-allele carriers was not affected by their physical activity levels. These authors hypothesized that physical activity may help CC homozygotes to achieve a lower BMI, as they found that physically passive CC homozygotes had a higher BMI than the physically active CC subjects (Andreasen *et al*. 2008). The discrepancy in the results could be explained by the fact that different study designs and interventions were used and subjects from different age groups and from different weight categories were involved. Franks *et al*. (2008) also indicated that various factors could be responsible for the disparate findings reported in their study compared to those on obese children (Reinehr *et al*. 2008, Reinehr *et al*. 2009), including the possibility that the results of both or one of these studies are false-positives.

While manipulation of physical activity levels based on the genotype of an individual may be considered as part of weight management interventions, the effect of dietary intake, especially fat and energy intake on energy balance, within the context of genotype, may also need to be considered. Animal studies have illustrated that INSIG mRNA expression is nutritionally regulated and is increased in response to high fat or energy diets (Li *et al*. 2002, Li *et al*. 2003) or is decreased in response to food restriction (Li *et al*. 2003). Although no association between the intake of high fat foods and energy-dense snacks and the *INSIG2* genotypes was found in our study, significant associations between these genotype groups and eating behaviour indicators that may result in a higher fat and energy intake, were found. The mutant CC subjects had a lower attitude to self-regulation (subscale of dietary restraint) score, which reflects poorer self-regulation of food intake. Furthermore, a modelled increase in the situational susceptibility to disinhibition score (reflects an increased tendency to overeat at social occasions, when food tastes good and when in the company of someone who overeats) was associated with the most pronounced increase in BMI in the mutant CC homozygotes. As no such association was found for the GG homozygotes and CC subjects were found to be more likely to have a poorer self-regulation of food intake, it can be speculated that this could result in CC subjects being more likely to consume excessive amounts of food, possibly higher in total fat and energy. This may result in a more pronounced increase in BMI of CC subjects, which is supported by the previously mentioned possibility that the mutant C-allele may result in a variant INSIG2 protein that is unable to block certain lipogenic enzymes, consequently leading to increased preadipocyte differentiation and lipogenesis in the presence of a high fat and energy diet. No previous reports investigating the effect of the *INSIG2* rs7566605 polymorphism on eating behaviour could be traced and our results therefore need to be confirmed by futher research.

In summary, our results indicate that the mutant CC homozygotes of the INSIG rs7566605 polymorphism may have a higher risk to develop MetS as they had lower HDL levels and a higher number of traits that met the diagnostic criteria of MetS. It is argued that our results are supported by the higher BMI in CC subjects found by others as well as the effect of a possible variant INSIG protein on changes in its physiological

function. Although the C-allele carriers had higher leisure-time physical activity levels, it was evident that the GG homozygotes could possibly decrease their BMI by increasing their leisure-time physical activity levels. Three of the published physical activity/weight loss intervention studies that included physical activity components support this possibility, while two refute it. We therefore suggest that although there are indications that the INSIG2 protein may be involved in MetS and the interaction between physical activity and BMI, more evidence supporting our findings is necessary before genotype specific recommendations can be made in this regard. Novel findings that warrant further investigation include the findings that mutant CC homozygotes have a lower attitude to self-regulation score and they may be more likely to experience an increase in BMI as their situational disinhibition score increases. It is argued that the proposed changes in the physiological functioning of a possible variant INSIG2 protein may result in an inability to block lipogenesis, which could be to the disadvantage of CC subjects who are more likely to exhibit poor eating behaviours, which may result in increase fat and energy intakes and consequently leading to a higher BMI.

The FTO rs1421085 and rs17817449 polymorphisms

The significant associations that were found for the two *FTO* polymorphisms involved perceived weight history, MetS traits, physical activity levels, food intake, a number of eating behaviour indicators and indicators of depression and general psychological well-being.

It was evident from our study that fewer subjects with the mutant CC genotype of the *FTO* rs1421085 polymorphism thought they were overweight during adolescence, while there were no differences between genotypes for perceived weight during childhood or young adulthood and for current weight. If it is assumed that perceived weight is a proxy for actual weight, these results are in contrast with published trends that indicate consistently that the C-allele is associated with a higher BMI and with early-onset obesity in Caucasians (Dina *et al*. 2007b, Scuteri *et al*. 2007, Attaoua *et al*. 2008, Do *et al*. 2008, Peeters *et al*. 2008, Price *et al*. 2008, Cauchi *et al*. 2009, Stutzmann *et al*. 2009). However, it must be borne in mind that even though perceived weight has been suggested to be a valid proxy for actual weight (Munoz *et al*. 1996, Koprowski *et al*. 2001, Must *et al*. 2002), it may not have been sensitive enough to detect genotype differences regarding weight history for this polymorphism. It is also possible that this result is a false positive.

Associations between the two *FTO* polymorphisms and MetS traits were found for HDL. For the homozygous mutant subjects of the two *FTO* polymorphisms as well as the mutant C-G haplotype a modelled decrease in BMI was associated with an increase in HDL. It can thus be hypothesized that weight loss may help overweight/obese homozygous mutant subjects to achieve higher HDL levels. No previous studies could be traced that reported on the association between MetS or lipid profile and these two *FTO*

polymorphisms in Caucasians. However, the mutant allele carriers of both polymorphisms have been associated with higher LDL and triglyceride levels in Asians (Tan *et al*. 2008). Research has shown that *FTO* is expressed inside the cell nucleus of almost all human tissues including adipocytes (Dina *et al*. 2007b). Although the exact physiological role of the FTO protein is still under investigation (Gerken *et al*. 2007, Fischer *et al*. 2009), recent research indicates that *FTO* probably encodes a 2-OG dependent nucleic acid demethylase. It is known that 2-OG dependent oxygenases are involved in DNA repair, fatty acid metabolism, and posttranslational modifications (Hausinger 2004, Clifton *et al*. 2006). The results of *in vitro* experiments indicated that *FTO* possibly exert gene regulation at RNA level in humans (Gerken *et al*. 2007, Jia *et al*. 2008). Furthermore, as a 2-OG dependent oxygenase it might play a potential role in adaptation to hypoxia and lipolysis (Gerkin *et al*. 2007, Sanchez-Pulido & Andrade-Navarro 2007).

According to Frayling *et al*. (2007) the highest levels of *FTO* expression are found in the brain, specifically in the arcuate nucleus of the hypothalamus, which is known to play a major role in controlling energy homeostasis and eating behaviour. Studies performed on humans, mice and rodents have shown that *Fto* mRNA expression is nutritionally regulated (Gerken *et al*. 2007, Fredriksson *et al*. 2008, Stratigopoulos *et al*. 2008, Wahlen *et al*. 2008, Zabena *et al*. 2009) and that it is possibly involved in the regulation of energy expenditure (Stratigopoulos *et al*. 2008, Fischer *et al*. 2009). As eating behaviour and resulting dietary intake as well as physical activity are major factors in determining energy homeostasis, it is a matter of course that the association between these variables and the *FTO* polymorphisms be investigated. Our research showed clearly that such associations are prevalent. It must be borne in mind that the exact physiological explanations for these associations still need to be elucidated.

We found that a modelled increase in the sport index score was associated with a significant decrease in the BMI of subjects with the wild-type T-T haplotype compared to the C-T haplotype. This implies that subjects with both wild-type alleles of the two *FTO* polymorphisms could benefit more from increasing their physical activity levels to lose or maintain weight. It can also be speculated that mutant allele carriers may find it more difficult than the wild-type T-T carriers to manage their weight or to lose weight when their physical activity levels are similar. Therefore, if mutant allele carriers employ physical activity as a weight loss strategy, they would need to train harder than subjects with the wild-type haplotype. If this is not possible, they would need to consider restricting their energy intake more than the wild-type haplotypes. The notion that mutant allele carriers may need additional increases in physical activity levels or decrease energy intake more strictly compared to subjects with the wild-type haplotype in order to maintain or lose weight, may explain current findings that the mutant alleles of both *FTO* polymorphisms have consistently been associated with a higher BMI or obesity prevalence (Dina *et al*. 2007b, Scuteri *et al*. 2007, Attaoua *et al*. 2008, Do *et al*. 2008, Peeters *et al*. 2008, Price *et al*. 2008, Cauchi *et al*. 2009, Stutzmann *et al*. 2009).

A number of significant associations between the two *FTO* polymorphisms and food intake, eating behaviour as well as psychological health of overweight/ obese subjects were found in this study, more so than for any of the other polymorphisms tested. A modelled increase in the attitude to self-regulation score (subscale score of dietary restraint, reflecting a better eating behaviour) of wild-type TT subjects of the *FTO* rs1421085 polymorphism was associated with a decrease in BMI. These subjects may thus benefit from increasing their attitude to self-regulation if they attempt to achieve a lower BMI, however the same beneficial effect may not be experienced by the mutant allele carriers. The mutant CC subjects of the *FTO* rs1421085 polymorphism may further be challenged by the fact that a modelled increase in the rigid control score (subscale score of dietary restraint) was associated with an increase in BMI. Higher rigid control reflects an all-or-nothing approach to dieting, with "fattening foods" or high energy containing foods being totally avoided. This type of dieting is very difficult to maintain over the long-term, often resulting in frequent lapses or total relapse, thus increased intake of fattening foods. It is thus not surprising that other researchers have found a positive association between rigid control scores and BMI, as well as a higher total energy intake and less success with weight loss in those with high scores (Westenhoefer *et al*. 1999).

Other significant associations from our study point to the possibility that the mutant C-allele carriers of the *FTO* rs1421085 polymorphism may have poorer eating behaviours. The mutant CC subjects of the *FTO* rs1421085 polymorphism were characterized by a higher hunger score, which reflects a higher susceptibility to general subjective feelings and perceptions of hunger which may result in higher food intake. Subscale analysis of the hunger score revealed that CC subjects' hunger was internally interpreted and regulated (higher internal locus for hunger score). This implies that CC subjects perceive to be hungry more often, resulting in eating more frequently during the day, often finding themselves "just having to eat something at anytime". The C-allele carriers were also characterized by a higher emotional disinhibition score, which is associated with experiencing disinhibition due to emotional feelings such as loneliness, anxiousness or feeling depressed (Bond *et al*. 2001).

However, in contrast to these results the mutant CC genotype was also associated with having a higher flexible control (subscale score of dietary restraint), which indicates a better approach to dieting and thus a better eating behaviour. Subjects with a higher score eat foods perceived to be fattening in small amounts, but without feelings of guilt and thus without losing control over food intake (Westenhoefer *et al*. 1999). Furthermore, it was also evident that the C-allele carriers had a lower intake of take-away foods compared to TT subjects.

When considering the interaction between food intake, eating behaviours and the *FTO* rs17817449 polymorphism the results support those of the *FTO* rs1421085 polymorphism that indicate that the mutant allele carriers may have poorer eating behaviours. The mutant GG subjects of the *FTO* rs17817449 polymorphism were characterized by higher scores for habitual susceptibility to disinhibition (disinhibition subscale) and rigid control (subscale score of dietary restraint). Habitual susceptibility to disinhibition refers to the fact that specific circumstances may predispose GG subjects to experience repeated disinhibition. For instance, when such a subject eats food that is not allowed on a diet, they could experience it as a lapse resulting in binging on other high energy containing foods. Such subjects are also likely to eat appropriate "healthy" foods in the presence of others, while splurging when on their own. They may also engage in eating binges when they are not hungry (Stunkard & Messick 1985). All in all these subjects may find it particularly difficult to diet due to these lapses. Typical dieting behaviour in this type of subject would involve starting to diet in the morning, but discontinuation thereof at some point in the day, therefore starting again the next day (Stunkard & Messick 1985). It must also be borne in mind that these results were dependent on gender as the associations disappeared when analyses were adjusted for gender. However, gender-adjusted results did indicate that the mutant GG subjects had a higher intake of high fat foods, which may be due to poorer eating behaviours.

Only one report (Stutzmann *et al*. 2009) investigating the eating behaviour related associations with the *FTO* rs1421085 polymorphism could be found, while none have been published for the *FTO* rs17877749 polymorphism. In the work by Stutzmann *et al*. (2009) no associations were found between the *FTO* rs1421085 polymorphism and the scores of the restraint, disinhibition or perceived hunger scales of the TFEQ in obese and non-obese French adults. These authors did not investigate associations with subscale scores of these three eating behaviours as was done in our research. Stutzmann *et al*. (2009) also did not find any associations between the *FTO* rs1421085 polymorphism and snacking or eating large quantities of food at meals in Swiss class III obese adults, or with snacking and bulimia in French adults from a randomly selected population sample. However, our results, as described above, provide support for the notion that the *FTO* gene may be associated with food intake and the regulation of eating behaviour (Speakman *et al*. 2008, Wardle *et al*. 2009). Furthermore, results from studies that investigated the associations between dietary intake or eating behaviours and other *FTO* polymorphisms that are located in the same LD block as the rs1421085 and rs17877749 polymorphisms (Dina *et al*. 2007b, Frayling *et al*. 2007, Scuteri *et al*. 2007) seem to support our findings that the mutant alleles of these two polymorphisms may be associated with poorer eating behaviours. Wardle *et al*. (2008) showed that the mutant genotype or allele of the *FTO* rs9939609 polymorphism in Caucasian children was associated with impaired ability to respond to satiety cues (Wardle *et al*. 2008), a higher intake of highly palatable foods (Wardle *et al*. 2009) and a higher energy intake (Cecil *et al*. 2008). These children also experienced more frequent "loss of control" eating episodes and ate food that is higher in fat at a lunch buffet (Tanofsky-Kraff *et al*. 2009). Although the majority of

studies did find positive associations involving the *FTO* rs9939609 polymorphism, one study was unable to replicate these results in children (Hakanen *et al*. 2009). In Caucasian adults a higher energy intake was also reported for mutant allele carriers of the *FTO* rs9939609 (Speakman *et al*. 2008) and rs8050136 polymorphisms (Haupt *et al*. 2009). From the aforementioned discussion it can be concluded that there is some support for our findings that mutant allele carriers of the *FTO* polymorphisms may have poorer eating behaviours (reflected by higher hunger, internal locus for hunger, emotional disinhibition, habitual disinhibition and rigid control scores). Within this context it could be possible that our results indicating that the mutant genotype has a higher flexible control and lower intake of take-away foods (reflecting better eating behaviours) are false positives as no support for this could be found. It is important to note that although a number of researchers have been investigating the associations between the FTO polymorphisms and physical activity, eating behaviour and dietary intake the exact physiological mechanisms involved are still unknown.

The current study also suggests that the mutant GG subjects of the *FTO* rs17877749 polymorphism have more depressive symptoms (as measured by the BDI). Furthermore, a modelled increase in psychological distress (as measured by the GHQ) was associated with a more pronounced increase in the BMI of the wild-type TT subjects of the *FTO* rs1421085 polymorphism. It is known that various mental and personality disorders including mania, anxiety and depression contribute to the hedonic aspects of overeating and may predict the development of obesity in both men and women (Ahlberg *et al*. 2002, Davis 2009). No other reports could be traced that investigated the association between *FTO* polymorphisms and psychological health.

In summary, although the exact physiological function of FTO is still unknown, it is has been recognized that it may play an important role in the regulation of food intake, eating behaviours and energy expenditure (physical activity), which does support the plausibility of the results of this research. The wild-type T-T haplotype of the two *FTO* polymorphisms investigated in this study experienced the most pronounced decrease in BMI in response to an increase in the sport index score. The mutant allele carriers or mutant homozygotes of either the *FTO* rs1421085 or rs17817449 polymorphisms were associated with a higher intake of high fat foods as well as poorer eating behaviours, reflected in higher scores for perceived hunger, internal locus for hunger, emotional and habitual susceptibility to disinhibition and rigid control of dietary restraint. Furthermore, an increase in rigid control was also associated with the most pronounced increase in the BMI of mutant homozygotes of the *FTO* rs1421085 polymorphism. For the wild-type homozygotes of the *FTO* rs1421085 polymorphism the most pronounced decrease in BMI was found for a modelled increase in self-regulation. These results are supported to some extent by associations found between food intake or eating behaviour indicators and *FTO* polymorphisms that are in strong LD with the two FTO polymorphisms investigated in this research.

Other novel findings that warrant further investigation include: 1) the wild-type T-allele carriers of the *FTO* rs17817449 polymorphism had fewer depressive symptoms; 2) the wild-type TT homozygotes of the *FTO* rs1421085 experienced the most pronounced increase in BMI in response to increase in psychological distress and 3) a modelled increase in BMI was associated with the most pronounced decrease in HDL levels of the mutant homozygotes of both polymorphisms, which was confirmed with haplotype analysis.

The ADRB2 Arg16Gly polymorphism

The only significant associations that were found for the *ADRB2* Arg16Gly polymorphism involved perceived weight history and an eating behaviour related indicator.

If it is assumed that perceived weight history is a proxy for actual weight history (Munoz *et al*. 1996, Koprowski *et al*. 2001, Must *et al*. 2002), our results indicate that the wild-type Arg16Arg genotype may predispose such individuals to being overweight during childhood, while Gly16-allele carriers were less likely to be overweight during childhood. However, these associations disappeared during adolescence and young adulthood. There was also no association between this polymorphism and actual BMI at the time of the study. It can thus be speculated that the latter outcomes (no differences between genotype groups and actual weight at the time of this study or perceived weight during adolescence and young adulthood) resulted from more weight gain in the Gly16-allele carriers than Arg16Arg homozygotes during adolescence and early adulthood. This is supported by studies that found a higher frequency of the Gly16Gly genotype in Caucasian men (aged 20-40 years old) who gained weight over a seven year period (Van Rossum *et al*. 2002) as well as in Japanese overweight/obese men (aged <50 years old) who gained weight over a five year period (Kawaguchi *et al*. 2006) compared to their respective weight stable Caucasian and Japanese counterparts. Further support for increased sensitivity to weight gain in Gly16-allele carriers comes from childhood and teenage cohort studies in Caucasian and African Americans. Although no significant associations between the *ADRB2* Arg16Gly polymorphism and the weight of newborns and children were found, the Gly16-allele carriers gained more weight per annum, which consequently resulted in a higher BMI during young adulthood (Ellsworth *et al*. 2002). This is also in line with studies that indicate positive associations between the Gly16-allele carriers or Gly16Gly homozygotes and a higher BMI in Caucasians (Ehrenborg *et al*. 2000, Meirhaeghe *et al*. 2001) and Brazilians (Mattevi *et al*. 2006). However it must be borne in mind that these genotypes have also been linked with a lower BMI in Caucasians (Ukkola *et al*. 2000) and that the balance of evidence indicates that there is no association between the *ADRB2* Arg16Gly polymorphism and BMI or obesity (Large *et al*. 1997, Meirhaeghe *et al*. 2000, Oberkofler *et al*. 2000, Rosmond *et al*. 2000, Terra *et al*. 2005, Gjesing *et al*. 2007, Lima *et al*. 2007).

As far as eating behaviour related indicators are concerned, it was found that a modelled increase in the flexible control score (subscale of dietary restraint) was associated with a more pronounced decrease in the BMI of Arg16Arg homozygotes. This decrease in BMI was less pronounced in the heterozygotes and non existent in the mutant Gly16Gly homozygotes. These results support the possibility that a focus on improving flexible control could help Arg16Arg subjects to achieve a lower BMI or could help them to be more effective weight managers and prevent weight gain in the long-term. The fact that a modelled improvement in flexible control did not affect the BMI of the mutant Gly16Gly homozygotes in this study may indicate that these subjects may find it more difficult to lose weight than the wild-type Arg16Arg homozygotes. The work by Masuo *et al*. (2005) possibly supports this notion. They found that a two year intervention consisting of a low fat diet, exercise and nutrition education and behaviour sessions resulted in a significant reduction in the weight of Arg16Arg homozygotes, while Gly16-carriers were resistant to weight loss (Masuo *et al*. 2005). Although eating behaviour indicators were not assessed, it can be speculated that the findings from the latter study support a focus on flexible control in Arg16Arg homozygotes, as a higher flexible control is necessary to achieve and maintain a weight loss over a long-term period such as two years. It can also be argued that these results stem from the changes that occur in the physiological functioning of ADRB2 due to the Arg16Gly polymorphism. It has been shown that the Gly16-allele is associated with a higher decrease in ADRB2 expression in adipose tissue due to enhanced down-regulation after prolonged agonist stimulation (Green *et al*. 1994, Green *et al*. 1995). This may consequently result in reduced receptor function and lower efficiency of lipolysis stimulation, leading to excess fat accumulation over time (Ellsworth *et al*. 2002).

In summary, the perceived weight results from this research indicate that Arg16Arg homozygotes were more likely to think that they were overweight during childhood. As no associations between this polymorphism and perceived weight during adolescence or young adulthood or with weight measured at the time of this study were found, it is speculated that Gly16Gly homozygotes may be more inclined to gain weight during the latter two life-cycle stages. As the literature in this regard is still equivocal, further research is necessary to confirm this association. The novel finding that Arg16Arg homozygotes may respond better to weight loss or weight management interventions that place emphasis on improving their flexible control of dietary restraint, warrants further investigation.

The GNB3 C825T polymorphism

For the *GNB3* C825T polymorphism several significant associations were found between the genotype groups and the investigated variables, including perception of weight, MetS traits, physical activity, dietary intake, depression and self-esteem.

The CT heterozygous subjects of the *GNB3* C825T polymorphism were more likely to perceive themselves to be overweight during childhood compared to the CC or TT homozygous subjects. These differences were not evident during adolescence or young adulthood. There was also no association between the polymorphism and actual BMI of subjects at the time of this study. As no evidence in the literature seems to support this association, the possibility that this is a false positive result need to be considered.

Assessment of associations with MetS traits revealed that the mutant TT homozygotes were more at risk to develop MetS than the CC homozygotes or C-allele carriers. The TT homozygotes had higher fasting triglyceride, glucose levels, a higher number of traits that met the diagnostic cut-off criteria for MetS and higher number of these subjects was diagnosed with MetS. These findings are in line with associations reported for the TT homozygous genotype with higher fasting glucose and insulin levels, HbA1c, insulin resistance and Type 2 diabetes prevalence in other Caucasian samples (Poch *et al*. 2002, Brand *et al*. 2003, Wascher *et al*. 2003, Andersen *et al*. 2006).

When considering the physical activity results, it was evident that a modelled increase in leisure-time activity resulted in a more pronounced decrease in the BMI in wild-type CC homozygotes. This association was less pronounced in the CT heterozygotes and non-existent in the mutant TT homozygotes. This finding is not supported by the results from two published studies conducted in Caucasian samples, namely that the interaction between the *GNB3* C825T polymorphism and physical activity levels has no effect on the risk to develop obesity (Grove *et al*. 2007) or on body composition changes after a 20-week endurance exercise programme (Rankinen *et al*. 2002). However, significant associations have been reported for non-Caucasian samples. Rankinen *et al*. (2002) reported greater decreases in fat mass and percentage body fat after the 20-week endurance exercise programme in African-American TT homozygotes (Rankinen *et al*. 2002). Grove *et al*. (2007) pointed out that TT-subjects with low physical activity levels had the highest risk for obesity development, while TT subjects with high physical activity levels had the lowest risk for obesity development (Grove *et al*. 2007). The results regarding the association between this polymorphism and physical activity levels are clearly still equivocal.

In terms of dietary intake, the CC subjects had a higher intake of high fat foods and energy-dense drinks. Furthermore, a modelled increase in the intake of energy-dense snacks was associated with a more pronounced increase in the BMI of CC subjects than the CT heterozygotes, while no such association was found in TT subjects. No other studies could be traced that investigated the association between this polymorphism and dietary intake specifically. It is thus possible that CC subjects may benefit more from decreased intake of high fat foods, energy dense snacks and energy-dense drinks, but further research is essential to confirm this outcome.

The associations between the *GNB3* C825T polymorphism and MetS traits, physical activity and dietary intake indicators can possibly be explained on the basis of the changes in the physiological function of the GNB3 protein associated with the C825T polymorphism. The *GNB3* gene encodes a β-subunit of the guanine nucleotide binding proteins (G-proteins) (Milligan & Kostenis 2006, Oldham & Hamm 2006). G-proteins are located on the cell membranes of WAT where they transduce signals that stimulate lipolysis in the adipocytes (Robidoux *et al*. 2004). The 825T-allele of the *GNB3* polymorphism has been associated with the expression of an altered G-protein (Siffert *et al*. 1998, Rosskopf *et al*. 2003a, Rosskopf *et al*. 2003b), decreased G-protein content in adipocytes (Rydén *et al*. 2002) as well as decreased catecholamine induced lipolysis in adipose tissue (Hauner *et al*. 2002, Rydén *et al*. 2002). The lower GNB3 protein levels in adipocytes as well as the decreased stimulation of lipolysis in TT subjects may explain why CC subjects respond better to physical activity or decreased intake of energy-dense snacks to lose weight, as normal lipolysis probably occurs in the CC subjects. Normal lipolysis stimulation may also explain the more favourable MetS profile found in the CC subjects. It can thus be speculated that TT subjects would need to have higher physical activity levels or a lower energy intake than CC subject in order to maintain weight or to achieve a negative energy balance and lose weight. However, more research is required to elucidate the exact physiological function of the *GNB3* splice variants and how this interacts with lifestyle variables and contributes to the development of obesity (Vogler *et al*. 2008).

The link between the *GNB3* polymorphism and psychological well-being has also been investigated by others because of the physiological role of the GNB3 protein in serotonin reuptake (Simon *et al*. 1991). Our results indicate that the mutant TT genotype experienced fewer depressive symptoms and had a higher self-esteem compared to the CC genotype or C-allele carriers. In contrast to our results, a meta-analysis of three case-control studies (Lopez-Leon *et al*. 2008) as well as research on Caucasian (Exton *et al*. 2003), Korean (Lee *et al*. 2004) and Chinese (Cao *et al*. 2007) samples found an association between the T-allele and having either depression or more depressive symptoms. However, others reported no association between depression and this polymorphism in Asian samples (Lin *et al*. 2001, Kunugi *et al*. 2002). Several studies also investigated the influence of the *GNB3* C825T polymorphism on antidepressant response in patients with major depressive disorder. Some indicate no association in Korean (Kang *et al*. 2007) and Japanese (Kato *et al*. 2008) samples, while others find a lack of response to specific anti-depressants in T-allele carriers (Wilkie *et al*. 2007, Lin *et al*. 2009). Most of these studies were performed in Asian populations and although they do contribute to the understanding of the association between the *GNB3* polymorphism and depression, more research is necessary to clarify these associations in Caucasians.

In summary, our results indicate that the mutant TT homozygotes had higher triglyceride and glucose levels, a higher number of traits that met the diagnostic cut-offs for MetS and a higher number of subjects were diagnosed with MetS. These findings are in line with other published reports in Caucasian populations. A

modelled increase in physical activity levels was associated with the most pronounced decrease BMI of the wild-type CC subjects. Support for this in the literature is conflicting and more research is necessary to clarify the association in Caucasian populations. The novel findings for the associations between the *GNB3* C825T polymorphism and dietary intake indicate that CC subjects had a higher intake of high fat foods and energy-dense drinks and that a modelled decrease in the intake of energy-dense snacks is associated with the most pronounced decrease in BMI of CC subjects. The associations with MetS, physical activity and dietary intake can be explained to some extent by the known effect of the *GNB3* C825T polymorphism on the physiological functioning of the GNB3 protein. The finding that TT subjects in our study had less depressive symptoms and a higher self-esteem is not in line with previous reports that there is either no association or an association between the T-allele and depression. As most of the research on psychological health involved Asian populations, more research is necessary to elucidate the association in Caucasian populations.

4.3 Conclusions

The results of this study in overweight/obese Caucasian adults with BMIs ranging from 27 to 52 kg/m^2 emphasize that the higher the BMI, the more compromised the physical health status of subjects, including increased MetS prevalence and diagnosed abdominal obesity, dyslipidemia (low HDL) and hypertension. As expected, a higher BMI was characterized by having lower sport and leisure-time physical activity levels; higher intakes of high fat foods, energy-dense snacks, take-away foods and energy-dense drinks; higher disinhibition, emotional disinhibition and habitual disinhibition, lower dietary restraint; and more depressive symptoms. The modelled relationships that were found between BMI and lifestyle and psychological health variables emphasize that these factors should form the foundation of a weight loss programme aimed at overweight/obese Caucasians.

The primary null hypothesis, namely that there is no association between genotype and BMI, could not be rejected. A number of the secondary and exploratory hypotheses were rejected (Table 4.16), thus reflecting associations between either genotype and variable and/or the effect of the interaction between genotype and variable on BMI. These results were supported by the literature in some instances, but some were also novel findings. A number of findings that are contradictory to the association in the literature or known physiological function were found (not included in Table 4.16), and the possibility that they are false positives needs to be considered.

Table 4.16: A summary of findings from this study

Findings from this study	Evidence: Novel/ supported	Physiological context link
***FABP2* Ala54Thr**		
❑ Mutant Thr54Thr: ↑ intake of take-away foods.	❑ Novel	❑ unknown
❑ Wild-type Ala54Ala: modelled decrease in BMI associated with decrease TG levels	❑ Novel	❑ Possible
❑ Wild-type Ala54Ala: ↓ self-esteem	❑ Novel	❑ unknown
***INSIG2* rs7566605:**		
❑ Mutant CC: ↑ MetS risk as they had ↓ HDL levels & more MetS traits	❑ Others found no association	❑ Possible
❑ Wild-type GG: ↓ leisure-time activity levels ❑ Wild-type GG: modelled ↑ leisure-time activity levels was associated with ↓ BMI	❑ Supported but equivocal findings	❑ Possible
❑ Mutant CC: lower attitude to self-regulation score & modelled ↑ in situational disinhibition score associated with ↑ in BMI	❑ Novel	❑ Possible
***FTO* rs1421085**		
❑ Mutant CC: modelled ↓ BMI associated with ↑ HDL levels	❑ Novel	❑ Unknown
❑ Mutant CC: ↑ perceived hunger, ↑ internal locus for hunger, ↑ emotional disinhibition, a modelled ↑ in rigid control resulted in a ↑ in BMI	❑ Novel, but supported by *FTO* SNPs in LD with this SNP.	❑ Possible
❑ Wild-type TT: modelled ↑ in attitude to self-regulation of dietary restraint ↓ BMI.	❑ Novel	❑ Possible
❑ Wild-type TT: modelled ↓ in general psychological well-being (GHQ) associated with ↓ BMI	❑ Novel	❑ Unknown
***FTO* rs17817449**		
❑ Mutant GG: ↑ intake of high fat foods, ↑ habitual susceptibility of disinhibition, ↑ rigid control (dietary restraint).	❑ Novel, but supported by *FTO* SNPs in LD with this SNP.	❑ Possible
❑ Mutant GG: ↑ depressive symptoms.	❑ Novel	❑ Unknown
❑ Wild-type T-T haplotype: modelled ↑ sport index score associated with ↓ BMI	❑ Novel	❑ Possible

Table 4.16: *(continued)*

Findings from this study	Evidence: Novel/ supported	Physiological context link
***ADRB2* Arg16Gly:**		
❑ Arg16Arg: more thought they were overweight during childhood, but not during adolescence/young adulthood & no association with current BMI. Possible that Gly16Gly homozygotes may be more inclined to gain weight during the latter two life-cycle stages	❑ Supported but equivocal findings	❑ Possible
❑ Arg16Arg: ↑ flexible control of dietary restraint was associated with ↓ BMI	❑ Novel	❑ Possible
***GNB3* C825T**		
❑ Mutant TT: higher MetS risk as ↑ glucose, ↑ triglycerides, ↑ number of MetS traits & more had MetS.	❑ Supported	❑ Possible
❑ Wild-type CC: ↑ leisure-time activity was associated with ↓ BMI	❑ Supported, but equivocal findings	❑ Possible
❑ Wild-type CC: ↑ intake of high fat foods, energy-dense drinks, ↓ intake of energy-dense snacks was associated with ↓ BMI	❑ Novel	❑ Unknown
❑ Wild-type CC: more depressive symptoms and lower self-esteem	❑ Contradicts findings in Asian samples	❑ Possible

Bearing in mind the limitations of the study (see Chapter 6, p250-251) it can be concluded that there was no association between genotype and BMI in this study sample. Furthermore, several associations between genotype and health and lifestyle related factors were prevalant, of which the most plausible associations are that the mutant TT homozygotes of the *GNB3* C825T polymorphism may have a higher risk to develop MetS and that the mutant alleles of the two *FTO* polymorphism are associated with poorer eating behaviours and higher intake of high fat foods.

Chapter 5

Intervention study

Results, discussion and conclusions

5.1 Results

Participants in the weight loss programme

Two-thirds (66%) of the 133 subjects included in the cross-sectional sample volunteered to participate in the six months weight loss programme. The subjects who chose not to participate in the programme (n=45) had a significantly higher mean±SD baseline weight (105.4±19.3 kg) than the n=88 subjects (97.0±20.1 kg) who did continue with the programme (p=0.0224). The baseline BMI (37.2±6.7 kg/m^2) of subjects who chose not to participate in the programme was also higher than the BMI (34.2±6.3 kg/m^2) of those who did continue with the programme (p=0.0135). No further significant differences between these two groups of subjects were found for any other variables, including physical activity, dietary intake, psychological health and eating behaviour.

Socio-demographic information

The subjects included in the weight loss programme had a mean±SD baseline age of 32.8±4.1 years, weight of 97.0±20.1 kg, height of 1.68±0.08 m and BMI of 34.2±6.3 kg/m^2. Just more than a quarter (26.1%) of these subjects was overweight (BMI of 27-29.9 kg/m^2), 38.6% were obese class I (BMI of 30-34.9 kg/m^2), 18.2% were obese class II (BMI of 35-39.9 kg/m^2) and 17.0% were obese class III (BMI ≥ 40 kg/m^2). Most were female, married, Afrikaans speaking, had a tertiary qualification and lived with their partners and children (Table 5.1). The males had a significantly higher baseline weight than the females, although the mean BMI was not different. There were no significant differences in mean BMI between the response categories of the socio-demographic variables.

Table 5.1: Socio-demographic profile and association with baseline weight and BMI

	n	%	Baseline weight mean±SD	p-value	Baseline BMI mean±SD	p-value
Gender						
Female	75	85.2	94.5±19.9	**0.0048**[†]	34.2±6.6	0.9156[†]
Male	13	14.8	111.3±15.1		34.4±4.5	
Marital status						
Married / living together	52	59.1	96.4±21.0	0.7158[†]	33.9±6.1	0.5175[†]
Unmarried (including separated/ divorced)	36	40.9	98.0±19.0		34.8±6.7	
Home language						
Afrikaans	78	88.6	96.2±19.6	0.2639[†]	34.0±6.1	0.2416[†]
English	10	11.4	103.7±23.4		36.5±7.5	
Level of education						
Completed high school	25	28.4	92.8±22.0	0.2248[§]	33.0±6.7	0.1055[§]
College/Technicon qualification	34	38.6	101.5±21.7		36.0±6.9	
University degree	29	33.0	95.4±15.6		33.2±4.7	
Living						
alone	18	20.5	100.2±20.4	0.7374[§]	35.5±6.9	0.8120[§]
with friends/ parents	14	15.9	96.6±19.6		34.1±6.7	
with a partner	19	21.6	99.3±20.1		33.6±5.1	
with a partner and child(ren)	37	42.0	94.5±20.6		34.0±6.6	

[†] Independent Samples t-test

[§] One-way ANOVA

Drop-outs

No significant differences between the drop-outs (n=47, 53%) and subjects who completed the full 24 weeks (n=41, 47%) of the study were found for the following cross-sectional assessments: age, height, weight, BMI and the scores of the physical activity, eating behaviour and psychological health questionnaires (Table 5.2). There were also no differences between weight and BMI measured at each follow-up point as well as mean perceived compliance scores of drop-outs (who were still in the programme at that specific follow-up point) and non-drop-outs. There were also no signifcant differences between subjects who remained in the four month active intervention phase of the programme but did not attend the six months follow-up (n=55, 63%) and drop-outs at four months (n=33, 37%) for all these baseline variables and follow-up weights and BMIs (results not included in a Table).

Table 5.2: Comparison of the mean±SD between the baseline data as well as weight, BMI and perceived compliance follow-up data of drop-out and non-drop-outs during the 24 week intervention period.

Variables	Non-drop-outs		Drop-outs		
	n*	Mean±SD	n*	Mean±SD	p-value†
Baseline age (years)	41	33.5±4.1	47	32.1±4.0	0.0925
Baseline height (m)	41	1.7±0.1	47	1.7±0.1	0.5361
Baseline weight (kg)	41	97.3±19.0	47	96.8±21.2	0.8956
Baseline BMI (kg/m^2)	41	34.5±5.5	47	34.0±7.0	0.7027
Perceived dietary compliance	27	5.6±1.5	29	4.9±2.1	0.2068
Perceived physical activity compliance	27	5.1±1.7	29	4.4±1.9	0.1253
Perceived behavioural compliance	27	5.0±1.5	29	4.4±2.1	0.2398
Total perceived compliance	27	5.2±1.2	29	4.6±1.8	0.1244
Baseline physical activity scores					
Work index	41	2.3±0.6	46	2.4±0.6	0.7058
Sport index	41	2.0±0.6	46	2.1±0.7	0.3871
Leisure-time index	41	2.0±0.6	46	2.0±0.5	0.8651
Total physical activity	41	2.1±0.4	46	2.2±0.4	0.4725
Baseline TFEQ scores					
Dietary restraint	41	8.6±3.8	45	7.9±4.6	0.4911
Disinhibition	41	10.7±3.7	45	9.7±3.7	0.2296
Perceived hunger	41	7.2±3.4	45	6.8±3.5	0.6160
Baseline scores of psychological health variables					
Beck depression inventory	41	13.9±11.0	46	13.2±8.1	0.7225
General health questionnaire	41	14.3±5.1	45	16.3±5.5	0.0944
Rosenberg self-esteem scale	41	19.8±5.8	46	19.2±4.9	0.5839

TFEQ = three-factor eating questionnaire

*n vary due to missing values

† Independent Samples t-test

Perceived compliance with the weight loss programme

The subjects' mean perceived compliance scores for each of the three components of the weight loss programme as well as the total perceived compliance score are presented in Table 5.3. These mean compliance scores were all around 50% (a score of 5 out of 10).

The correlation matrix that was constructed to assess the associations between compliance scores and weight variables indicates that the scores were not associated with baseline weight or BMI (Table 5.3). Weight and BMI changes from baseline to follow-up were also not associated with physical activity or behavioural compliance scores. However, higher weight and BMI losses were significantly associated with higher dietary compliance and total compliance scores.

Table 5.3: Mean±SD perceived compliance scores and correlation matrix of perceived compliance scores and baseline weight and BMI as well as change in weight and BMI overtime (24 weeks).

Perceived compliance variable	Compliance scores out of 10 Mean±SD	Correlation matrix	Baseline weight	Baseline BMI	Weight change (Baseline – Follow-up)	BMI change (Baseline – Follow-up)
Dietary compliance	5.2±1.8	r	-0.1107	0.0130	0.6157	0.6294
		p	0.416	0.924	**0.001**	**<0.0001**
Physical activity compliance	4.7±1.9	r	0.0537	0.1024	0.2548	0.2413
		p	0.694	0.453	0.200	0.225
Behavioural compliance	4.7±1.8	r	0.0061	0.0583	0.3479	0.3208
		p	0.965	0.670	0.075	0.103
Total compliance	4.9±1.6	r	-0.0193	0.0672	0.5131	0.5013
		p	0.888	0.623	**0.006**	**0.008**

Differences between Baseline and Follow-up weight and BMI

The subjects had a mean (SE) weight loss of 8.07 (0.72) kg ($p<0.0001$) and BMI loss of 2.92 (0.26) kg/m^2 ($p<0.001$) over the total study period of 24 weeks. This reflects a mean percentage weight loss of 8.6% over 24 weeks. The modelled time trajectories for weight and BMI measured every second week from baseline for the duration of the programme are depicted in Table 5.4. The difference between baseline and follow-up weight at 24 weeks was significant, whereas the difference between baseline BMI and follow-up BMI was significant at 14, 16 and 24 weeks.

Table 5.4: Unadjusted mean (SE) for weight and BMI change from baseline to 24 weeks.

Weeks in programme	n	Δ Weight (kg)		Δ BMI (kg/m^2)	
		Difference from baseline (standard error)	p-value[†]	Difference from baseline (standard error)	p-value[†]
2	83	-0.82 (3.08)	0.7908	-0.28 (0.95)	0.7665
4	80	-1.01 (3.11)	0.7464	-0.42 (0.96)	0.6624
6	70	-4.51 (3.22)	0.1625	-1.67 (0.99)	0.0922
8	69	-2.95 (3.24)	0.3625	-1.33 (0.99)	0.1809
10	62	-3.72 (3.34)	0.2655	-1.21 (1.03)	0.2375
12	57	-3.81 (3.42)	0.2659	-1.19 (1.05)	0.2566
14	48	-6.92 (3.61)	0.0559	-2.63 (1.11)	**0.0180**
16	55	-5.15 (3.46)	0.1366	-2.14 (1.06)	**0.0448**
24	41	-7.76 (3.81)	**0.0417**	-2.65 (1.17)	**0.0240**

*n reflects drop-out during intervention;

[†]Linear mixed-effects model with weeks as fixed effect and individual as random effect.

Differences between Baseline and Follow-up scores in physical activity, eating behaviour and psychological health variables

Significant changes from Baseline to the 16 week Follow-up assessment were evident for all the mentioned variables except for the work index score (Table 5.5). The changes included significant increases in the scores of the sport and leisure-time indices as well as the total physical activity score of the Baecke physical activity questionnaire, and significant improvements in the scores for depression (BDI), general psychological well-being (GHQ), self-esteem (RSES) and all the scales and subscales of eating behaviour (TFEQ) (Table 5.5).

Table 5.5: Mean (SE) difference in Baseline to 16-weeks Follow-up scores of physical activity, eating behaviour and psychological well-being variables.

	Variable (score range)	n*	Follow-up minus Baseline mean (standard error)	p-value†
Physical activity	Δ Sport index score	52	0.39 (0.10)	*0.0002*
	Δ Work index score	52	-0.02 (0.06)	0.7628
	Δ Leisure-time index score	52	0.38 (0.08)	*<0.0001*
	Δ Total physical activity score	52	0.25 (0.06)	*0.0001*
Eating behaviour	Δ Factor I: Dietary restraint (0-21)	51	4.96 (0.62)	*<0.0001*
	Δ Factor II: Disinhibition (0-16)	51	-3.22 (0.50)	*<0.0001*
	Δ Factor III: Perceived hunger (0-14)	51	-3.24 (0.45)	*<0.0001*
❑ **Dietary restraint subscales:**	Δ Flexible control (0-7)	51	1.67 (0.29)	*<0.0001*
	Δ Rigid control (0-7)	51	1.63 (0.25)	*<0.0001*
	Δ Strategic dieting (0-4)	51	0.96 (0.21)	*<0.0001*
	Δ Self-regulation (0-5)	51	1.29 (0.20)	*<0.0001*
	Δ Avoidance of fattening foods (0-4)	51	0.63 (0.19)	*0.0020*
❑ **Disinhibition subscales:**	Δ Habitual disinhibition (0-5)	51	-1.12 (0.22)	*<0.0001*
	Δ Emotional disinhibition (0-3)	51	-0.67 (0.16)	*0.0001*
	Δ Situational disinhibition (0-5)	51	-1.31 (0.20)	*<0.0001*
❑ **Perceived hunger subscales:**	Δ Internal locus (0-6)	51	-1.59 (0.25)	*<0.0001*
	Δ External locus (0-6)	51	-1.47 (0.24)	*<0.0001*
Psychological well-being	Δ Beck depression inventory (0-63)	52	-8.35 (1.34)	*<0.0001*
	Δ General health questionnaire (0-30)	51	-5.90 (1.00)	*<0.0001*
	Δ Rosenberg self-esteem scale (10-40)	52	1.54 (0.59)	0.0117

*n vary due to missing values; †Linear model of 16-week follow-up as a function of baseline variable

Genotype and allele frequencies

The genotype and allele frequencies of the 88 participants in the intervention are presented in Table 5.6. No mutant genotype was found in this sample for the *ADRB3* Trp64Arg polymorphism. The frequencies of the mutant alleles were higher than the wild-type alleles for the *ADRB2* Arg16Gly and FTO rs17817449 polymorphisms.

Although the genotype frequencies of the *GNB3* C825T and FTO rs17817449 polymorphisms were not in HWE, it must be borne in mind that all subjects were overweight/obese and not representative of a random population.

Table 5.6: Genotype and allele frequencies for overweight/obese Caucasian subjects in a weight loss programme

Gene	Polymorphism	Genotype frequency			Allele frequency		HWE
		Base pair	n*	%	Allele	%	p-value†
FABP2	Ala54Thr	GG (wild-type)	28	38.9	G-allele	58.3	0.0930
		GA	28	38.9	A-allele	41.7	
		AA	16	22.2			
INSIG2	rs7566605	GG (wild-type)	30	40.0	G-allele	66.0	0.2084
		GC	39	52.0	C-allele	34.0	
		CC	6	8.0			
FTO	rs1421085	TT (wild-type)	18	26.5	T-allele	52.9	0.8074
		TC	36	52.9	C-allele	47.1	
		CC	14	20.6			
FTO	rs17817449	TT (wild-type)	24	33.3	T-allele	48.6	0.0010
		TG	22	30.6	G-allele	51.4	
		GG	26	36.1			
ADRB3	Trp64Arg	TT (wild-type)	54	90.0	T-allele	95.0	1.0000
		TC	6	10.0	C-allele	5.0	
		CC	0	0			
ADRB2	Arg16Gly	AA (wild-type)	10	14.1	A-allele	40.8	0.4644
		AG	38	53.5	G-allele	59.2	
		GG	23	32.4			
GNB3	C825T	CC (wild-type)	31	44.3	C-allele	62.1	0.0449
		CT	25	35.7	T-allele	37.9	
		TT	14	20.0			

*n vary due to missing values

† R exact test

Effect of genotype on weight and BMI changes

Total compliance and gender-adjusted p-values for genotype and additive allelic effect on time trajectories (change in weight and BMI) for different follow-up periods from baseline are presented in Table 5.7.

Table 5.7: Total compliance and gender-adjusted p-values for genotype and additive allelic effect on time trajectories (change in weight and BMI) for different time periods from baseline.

Polymorphisms	p-values adjusted for gender and compliance			
	Δ BMI		Δ Weight	
	genotype[†]	additive allelic[§]	genotype[†]	additive allelic[§]
1st month				
FABP2 Ala54Thr	0.8446	0.5568	0.8372	0.5485
INSIG2 rs7566605	0.1910	0.0673	0.3218	0.1309
FTO rs1421085	0.7029	0.4681	0.5422	0.2977
FTO rs17817449	0.0619	0.7786	0.1197	0.8215
ADRB3 Trp64Arg	0.9913	0.9913	0.9519	0.9519
ADRB2 Arg16Gly	**0.0228**	**0.0114**	**0.0179**	***0.0087***
GNB3 C825T	0.2131	0.4134	0.2216	0.5508
2 months				
FABP2 Ala54Thr	0.9675	0.8123	0.9687	0.8127
INSIG2 rs7566605	0.3544	0.1893	0.3821	0.2132
FTO rs1421085	0.5394	0.2623	0.3507	0.1445
FTO rs17817449	**0.0398**	0.8549	0.0576	0.9730
ADRB3 Trp64Arg	0.8509	0.8509	0.8989	0.8989
ADRB2 Arg16Gly	0.1002	0.0754	0.0741	0.0503
GNB3 C825T	0.3648	0.2511	0.3765	0.2818
3 months				
FABP2 Ala54Thr	0.8338	0.6398	0.8099	0.5989
INSIG2 rs7566605	0.6771	0.3737	0.7333	0.4276
FTO rs1421085	0.9637	0.9532	0.8877	0.6773
FTO rs17817449	0.1903	0.6230	0.2531	0.6905
ADRB3 Trp64Arg	0.4883	0.4883	0.5526	0.5526
ADRB2 Arg16Gly	0.4281	0.2343	0.3203	0.1628
GNB3 C825T	0.7442	0.4377	0.8025	0.5070
4 months				
FABP2 Ala54Thr	0.7964	0.5727	0.7725	0.5435
INSIG2 rs7566605	0.6072	0.3148	0.6359	0.3378
FTO rs1421085	0.9933	0.9146	0.9819	0.8451
FTO rs17817449	0.3078	0.9372	0.3572	0.9890
ADRB3 Trp64Arg	0.4169	0.4169	0.4719	0.4719
ADRB2 Arg16Gly	0.4742	0.3000	0.3841	0.2320
GNB3 C825T	0.7241	0.5073	0.7875	0.5763
6 months				
FABP2 Ala54Thr	0.8879	0.6760	0.8598	0.6562
INSIG2 rs7566605	0.5969	0.3649	0.6326	0.3943
FTO rs1421085	0.9474	0.8771	0.9626	0.8972
FTO rs17817449	0.5305	0.9997	0.6180	0.9506
ADRB3 Trp64Arg	0.3618	0.3618	0.4253	0.4253
ADRB2 Arg16Gly	0.3838	0.2578	0.2969	0.2025
GNB3 C825T	0.7868	0.6697	0.8431	0.7742

[†]Linear model of genotype groups (categorised as wild-type, heterozygous and mutant genotypes) on BMI/ weight.

[§]Linear model of the additive allelic variable (numerically categorised as 0, 1 and 2 minor alleles) on BMI/ weight.

The gender-adjusted and total compliance adjusted genotype and additive allelic effect on weight and BMI time trajectories for the first four weeks on the weight loss programme was significant for the *ADRB2* Arg16Gly polymorphism (Table 5.7). This significant difference for weight change during the first month

(p=0.0087) is depicted in the slopes of the lines in Figure 5.1, which shows the modelled trajectories, from an additive allelic model, for a woman with an average compliance of 4.9, stratified by genotype. The modelled weekly weight loss for mutant Gly16Gly (GG) homozygotes is 0.38 kg for the first month, which is significant. For the heterozygotes (GA) the weekly loss is 0.28 kg more, thus 0.66 kg and for the wild-type Arg16Arg (AA) homozygotes it is 0.84 kg (a further additional 0.28 kg). Thus the mutant Gly16Gly homozygotes lost weight at a significantly slower rate than the heterozygotes whose weight loss was slower than that of the wild-type homozygotes (Figure 5.1). As the modeled additive allelic effect is the same for BMI, it was not depicted in a graph. The significant genotype effects are similar to the additive allelic effects for both weight and BMI, and are therefore also not depicted in separate graphs.

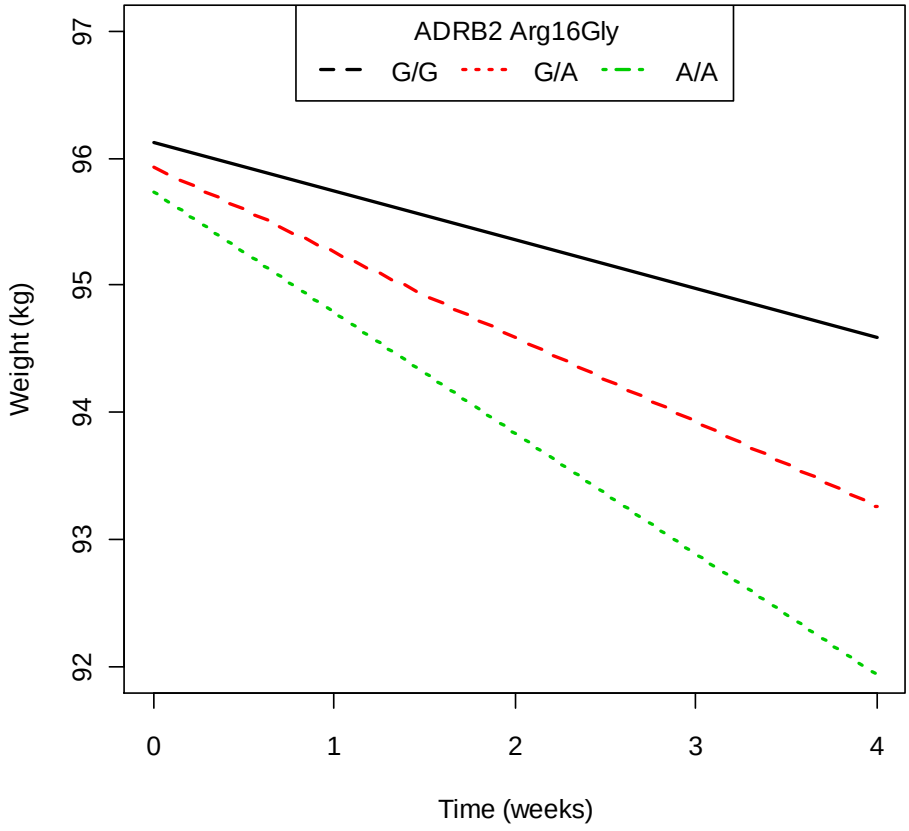

Figure 5.1: Plot of modelled weight against time (in weeks) for the first month on the weight loss programme. Lines show the modelled trajectories, from an additive allelic model, for a woman with average compliance of 4.9, for the genotypes of the *ADRB2* Arg16Gly polymorphism.

The gender and compliance adjusted genotype effect on BMI time trajectories for the first eight weeks on the weight loss programme was significant for the *FTO* rs17817449 polymorphism (Table 5.7). The modeled weight loss for the wild-type TT homozygotes was -1.67 kg during the first eight weeks of the programme, which is significantly more than the weight loss of -0.059 kg for GT or -0.175 kg for GG subjects (Figure 5.2). It is clear from Figure 5.2 that there is no additive allelic effect. It is also important to note that the additive allelic effect on weight change was not significant, therefore the possibility that the modeled significant effect on BMI change is a false positive result, needs to be considered.

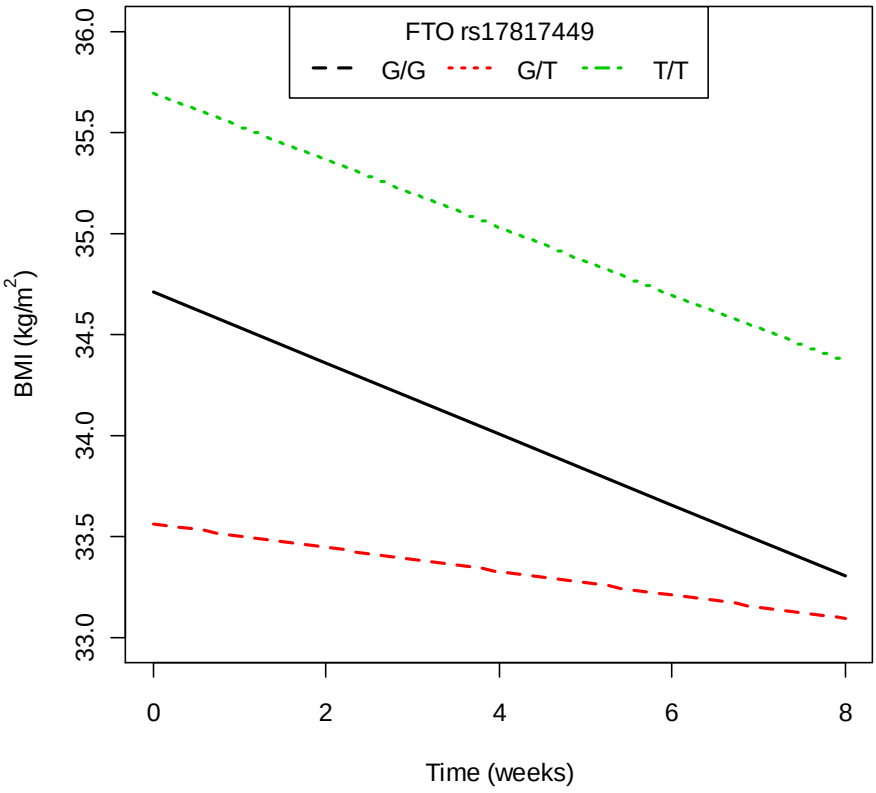

Figure 5.2: Plot of modelled BMI against time (in weeks) for the first two months on the weight loss programme. Lines show the modeled trajectories, from the genotype model, for a woman with average compliance of 4.9, for the genotypes of the *FTO* rs17817449 polymorphism.

None of the other polymorphisms had an effect on weight or BMI change during the first eight weeks of the weight loss programme. Furthermore, none of the polymorphisms had an effect on weight or BMI change from baseline to 12, 16 or 24 weeks (Table 5.7).

Effect of gene-gene interactions for all allelic combinations on Δ BMI

An interaction between the *ADRB2* Arg16Gly and *INSIG2* rs756605 polymorphisms had a significant (p=0.0028) effect on Δ BMI during the six month programme. Table 5.8 depicts the significant effects that

were found for all the allelic combinations of this gene-gene interaction. Each effect size presented in this table represents the average difference in Δ BMI for the specific allele combination compared to the reference combination which was G-G. The average difference in Δ BMI between subjects with both mutant alleles (G-allele of *ADRB2* and C-allele of *INSIG2*) and those with the G-G allelic combination is -2.6 kg/m^2. Thus the change in BMI for those with the mutant G-C alleles is -2.6 kg/m^2 less, on average, than for subject with the with G-G alleles. The average difference in Δ BMI between subjects with both wild-type alleles (A and G) is -1.59 kg/m^2 compared to subjects with the G-G alleles. The change in BMI for the A-C allelic combination does not differ significantly from the G-G allelic combination. None of the other gene-gene interactions were significant and therefore not included in the table.

Table 5.8: The effect of all allelic combinations of the gene-gene interaction between the *ADRB2* Arg16Gly and *INSIG2* rs7566605 polymorphisms on ΔBMI during the 24 week programme (gender and compliance adjusted).

***ADRB2* Arg16Gly** **A= wild-type allele** **G= mutant allele**	***INSIG2* rs7566605** **G= wild-type allele** **C= mutant allele**	**Allelic combination frequency**	**Effect size (kg/m^2)**	**SE**	**p-value**
A	C	0.19	-0.10	0.67	0.8798
A	G	0.20	-1.59	0.74	**0.0460**
G	C	0.11	-2.60	0.95	**0.0137**
G	G	0.51	haplotype base		

Effects of the interaction between Δ variable and polymorphism on Δ BMI

The gender and compliance-adjusted significant effects of the interactions between Δ variable (Baseline minus follow-up scores) and polymorphism on Δ BMI (Baseline minus follow-up BMIs) for the additive allelic model are illustrated in Figures 5.3 to 5.5.

The modelled rate of change in Δ BMI in response to a change in Δ emotional disinhibition score differed significantly between the genotype groups of the *FTO* rs1421085 polymorphism (Figure 5.3). The modelled rate of increase in BMI loss in response to an increased reduction in the emotional disinhibition score was more pronounced in the TT than TC subjects. As there were only a few mutant CC subjects and they had mostly a difference of zero or two on the emotional disinhibition score, the resulting modelled line in Figures 5.3 was not useful for interpretation purposes.

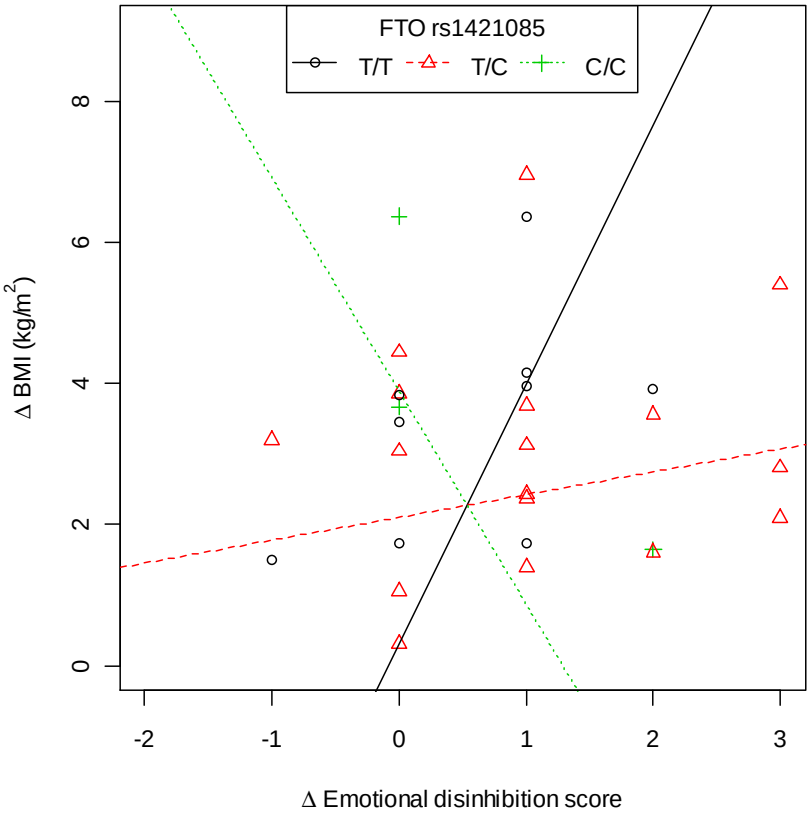

Figure 5.3: Plot of Δ BMI against Δ Emotional disinhibition score. Symbols represent individual observed values and regression lines show the expected relationships, from the additive allelic model, for a woman with average compliance of 4.9, for each genotype of the *FTO* rs1421085 polymorphism (p=0.0017). Δ = Baseline minus Follow-up values.

The rate of change in Δ BMI in response to a change in Δ BDI score differed significantly between the genotype groups of the *GNB3* C825T polymorphism (Figure 5.4). A modelled rate of increase in BMI loss over the 24 week intervention period in response to an increased reduction in BDI score was observed for the CC subjects but not for the CT subjects, while the association was in the opposite direction for the TT subjects.

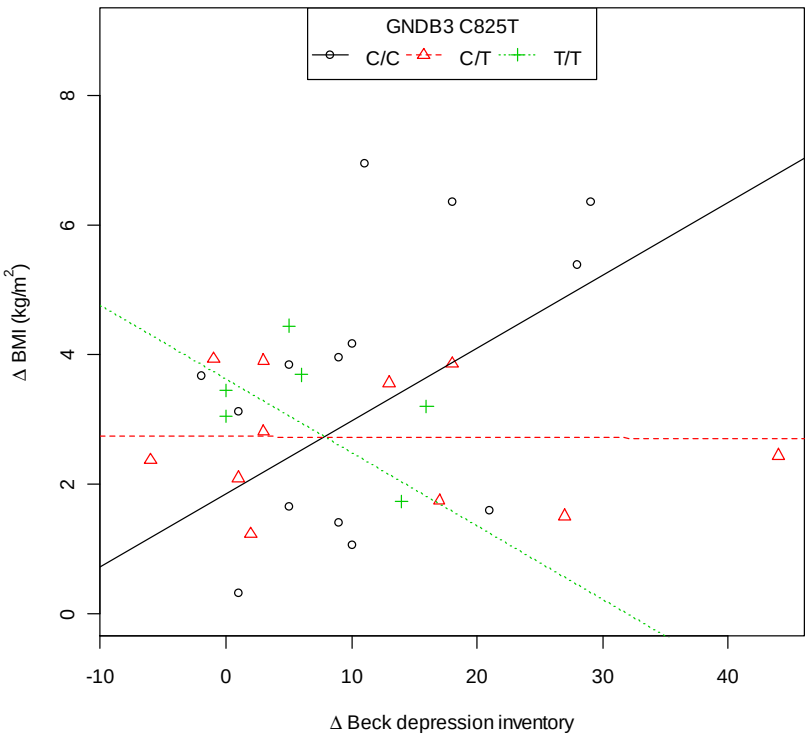

Figure 5.4: Plot of Δ BMI against Δ Beck depression inventory. Symbols represent individual observed values and regression lines show the expected relationships, from the additive allelic model, for a woman with average compliance of 4.9, for each genotype of the *GNB3* C825T polymorphism (p=0.0291). Δ = Baseline minus Follow-up.

The rate of change in Δ BMI in response to a change in Δ GHQ score differed significantly between the genotype groups of the *FABP2* Ala54Thr polymorphism (Figure 5.9). The modelled rate of increase in BMI loss over the 24-weeks intervention period in response to an increased reduction in the GHQ score was more pronounced in the mutant AA homozygotes than the heterozygotes, which was more pronounced than the wild-type GG homozygotes.

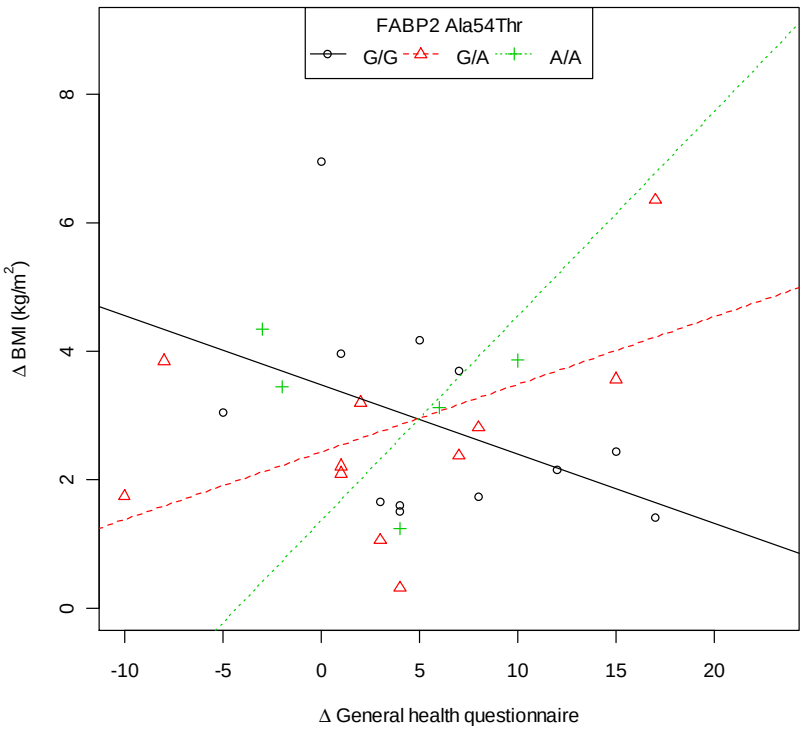

Figure 5.5: Plot of Δ BMI against Δ General health questionnaire. Symbols represent individual observed values and regression lines show the expected relationships, from the additive allelic model, for a woman with average compliance of 4.9, for each genotype of the *FTO* rs1421085 polymorphism (p=0.0261). Δ = Baseline minus Follow-up.

There were also significant differences between the modelled rate of change in Δ BMI in response to a change in the Δ work index score (p=0.0327), Δ disinhibition score (p=0.0331), Δ habitual disinhibition score (p=0.0150) and Δ avoidance of fattening food score (a subscale score of dietary restraint) (p=0.0238) for the *FTO* rs1421085 polymorphism. Figures for these results were not included as the exact nature (significant vs. non-significant) of the differences between the genotype groups are not evident from the genotype graphs.

5.2 Discussion

This discussion firstly focuses on the effectiveness of the conservative weight loss programme used in this study. Secondly, the influence of genotype on changes in weight loss treatment and process outcomes for each of the seven polymorphisms included in the study, are discussed.

Effectiveness of the weight loss programme

To investigate the effect of genotype on weight loss outcomes it was firstly important to establish whether the weight loss programme had been effective in inducing weight loss in the study sample. The energy

intake recommendations for weight loss were individually calculated for each subject included in this study to induce a 0.5 kg loss per week. This equates to an expected 12 kg weight loss over a six month time period. Although this is theoretically possible, the review of actual weight loss interventions by Franz *et al*. (2007) points to the fact that a lower mean weight loss over a six month period of 7.9 kg or 8.5% should be expected for interventions that include a diet and physical activity component and aim to achieve a 0.5 to 1 kg weight loss per week. As the mean significant weight loss in the current study of 7.8 kg or 8.6% is in line with the suggestions by Franz *et al*. (2007), we can conclude that our weight loss programme was effective in inducing significant and expected weight loss over the six months intervention period. The percentage weight loss also falls within the recommended range of 5 to 10% of initial body weight to ensure improvement in risk factors for comorbidities associated with excess weight (Seagle *et al*. 2009).

Significant changes in specific lifestyle and psychological health indicators that were the focus of strategies included in the programme, indicate that the success in terms of weight loss can be attributed to the strategies included in the programme. These changes include highly significant increases in leisure-time, sport and total physical activity levels and improvements in dietary restraint, disinhibition and perceived hunger as well as all subscale scores of these three eating behaviours. The subjects also experienced fewer depressive symptoms, a better general psychological well-being as well as a higher self-esteem. Such changes in physical activity levels, eating behaviours and psychological health have been noted as predictors of weight loss in obese patients on weight loss programme (Dalle Grave *et al*. 2009, Savage *et al*. 2009).

Attrition from the weight loss programme

It is important to consider the fact that there was a drop-out of 38% over the four months active intervention period and 53% for the six month follow-up assessments that could have resulted in bias in the data, although most weight-management intervention studies have to contend with the same problem (Pratt 1990, Yeh *et al.* 2003). To address this limitation time-trajectories were used to analyse the effect of genotype on weight loss outcome, with all individuals from the baseline sample (n=88) being included in the statistical models. It can also be argued that the bias in our study was limited by the fact that there were no significant differences between the genotype groups of each polymorphism as well as all other baseline variables between drop-outs and non-drop-outs. Furthermore, the final sample at four months (n=55) and six months (n=41) in our study is comparable to the samples that have typically been used in most of the 23 previous studies that investigated the effect of the polymorphisms included in this research on weight loss outcome. The sample size of 15 of these 23 studies were between 24 and 90, while four studies had samples between 111 and 185, three between 204 and 304 and one study included 3533 subjects. Despite the limitations imposed by the attrition rate, we feel that the results of this study make an important contribution to knowledge in the field of nutritional genomics, as very little in this regard has

been published on the *FABP2*, *INSIG2*, *ADRB2* and *GNB3* polymorphisms and no such information for the *FTO* polymorphisms included in this study has been published.

FABP2 Ala54Thr polymorphism

No associations were found between the *FABP2* Ala54Thr polymorphism and weight loss during the six month intervention period in the current study. These results are in line with the results from all previous studies that report on this association in Caucasian subjects after bariatric surgery (De Luis *et al.* 2008b) or after following weight loss interventions consisting of dietary, physical activity and behavioural components and lasting either two months (De Luis *et al*. 2008a), three months (De Luis *et al*. 2006), or six months in Japanese subjects (Takakura *et al*. 2005). Evidence from these studies does however, point to the possibility that the *FABP2* Ala54Thr polymorphism influences the outcome of other obesity phenotype indicators following a weight loss interventions. For instance, Ala54Ala homozygotes achieved a lower fat mass and leptin levels after three months (De Luis *et al*. 2006), lower leptin levels after two months (De Luis *et al*. 2008a) and a lower waist circumference after six months (Takakura *et al*. 2005) of intervention. However, it was also reported that the *FABP2* Ala54Thr polymorphism did not result in changes in waist circumference (De Luis *et al*. 2006, De Luis *et al*. 2008a) and fat mass (De Luis *et al*. 2008a, De Luis *et al*. 2008b) in Caucasian subjects following weight reduction. It is thus evident that although no association with weight or BMI loss is generally found, more specific phenotype indicators of body fat distribution and metabolism point to an association. The question can thus be asked whether BMI/weight is a sensitive marker for detecting changes in adiposity following weight loss interventions.

A novel finding from the current study indicates that an improvement in the psychological well-being (GHQ) of mutant Thr54Thr homozygotes over the first four months of the intervention period may have helped them to achieve a greater BMI loss over the total six months, compared to the other genotype groups. The currently known physiological functions of FABP2 do not provide a possible explanation for the association found with psychological well-being. It is thus possible that this result is a false positive and should thus be confirmed in further research.

INSIG2 rs7566605 polymorphism

No associations were found between the *INSIG2* rs7566605 polymorphism and weight loss during the six month intervention period in the current study. When considering gene-gene interactions it was found that subjects with a mutant C-allele of the *INSIG2* rs7566605 polymorphism and a mutant Gly16-allele of the *ADRB2* Arg16Gly polymorphism lost significantly less weight over the six month intervention period. This indicates that the C-allele carriers of the *INSIG2* rs7566605 polymorphism may be more resistant to weight loss on a conservative weight loss programme when they also have a mutant *ADRB2* allele.

When considering the effect of the *INSIG2* rs7566605 polymorphism on weight loss outcomes in the literature, most evidence points to the possibility that C-allele carriers are resistant to weight loss, while GG homozygotes are more likely to experience weight loss on a conservative weight loss programme. Two studies on independent samples of obese Caucasian children found that CC subjects lost less weight in the one year "Obeldicks" intervention programme that included nutrition education, physical exercise, behaviour therapy (covered in group sessions) and an individualized psychological care component (Reinehr *et al*. 2008, Reinehr *et al*. 2009). The results from a physical activity intervention in Caucasian men also indicated that GG subjects experienced a decrease in subcutaneous fat levels in their non-dominant arm following the 12-week resistance exercise intervention focused on this arm, while C-allele carriers were resistant to such fat losses (Orkunoglu-Suer *et al*. 2008). In contrast, Franks *et al*. (2008) found that overweight Caucasian adults with the CC genotype lost more weight than G-allele carriers after a one year lifestyle intervention as part of the Diabetes Prevention Programme. The discrepancy in the results could be explained by the fact that different study designs and interventions were used and subjects from different age groups and from different weight categories were involved. Franks *et al*. (2008) also indicated that various factors could be responsible for the disparate findings reported in their study compared to those on obese children (Reinehr *et al*. 2008, Reinehr *et al*. 2009), including the possibility that the results of both or one of these studies are false-positives.

FTO rs1421085 and rs17817449 polymorphisms

Although the function of FTO and its role in obesity development still needs to be elucidated, it has been proposed that FTO's expression in the hypothalamus (Dina *et al*. 2007b, Frayling *et al*. 2007, Gerken *et al*. 2007) is nutritionally regulated (Stratigopoulos *et al*. 2008) and that FTO is possibly involved in energy homeostasis by influencing energy expenditure (Fischer *et al*. 2009) as well as energy intake and eating behaviour (Speakman *et al*. 2008, Wardle *et al*. 2009). No previous reports on the association between the *FTO* rs1421085 and rs17817449 polymorphisms and weight loss outcomes could be traced. Two reports that focussed on another *FTO* polymorphism, namely *FTO* rs9939609 (also found in intron 1 of *FTO* and in strong LD with *FTO* rs1421085 and rs17817449), found no association between the polymorphism and weight loss outcomes in obese Caucasian children following the one year "Obeldicks" intervention programme (Müller *et al*. 2008) or in overweight American adults in the Diabetes Prevention Programme (DPP) (Franks *et al*. 2008). The finding in the current study that the wild-type TT homozygotes of the FTO rs17817449 polymorphism experienced a significantly greater reduction in BMI during the first two months of the intervention period compared to the heterozygotes or mutant homozygotes, is thus novel. This finding needs to be interpreted with care as it may be a false positive result as indicated in the results section and needs to be confirmed in further research.

It was evident that an improvement in emotional disinhibition in the wild-type TT subjects of the *FTO* rs1421085 polymorphism was associated with a more pronounced decrease in BMI over the six month weight loss period compared to CT subjects. Significant interaction effects for the **genotype** model were also found between the *FTO* rs1421085 polymorphism and changes in other eating behaviour scales (disinhibition, habitual disinhibition and avoidance of fattening foods [subscale of dietary restraint indicating better eating behaviour]) as well as changes in the work index scores on BMI losses during the six months intervention period. Although the latter results were not significant for the additive allelic variable thus only for the genotype model, it could be argued that it supports the possibility of FTO's involvement in the regulation of eating behaviours and warrants further investigations.

The results of this study therefore indicate that the wild-type homozygotes of the *FTO* polymorphisms might experience greater weight losses during the initial two months of a weight loss programme, while an improvement in eating behaviour may specifically help them to achieve greater weight losses over the total duration of the programme. These novel findings warrant further investigation.

ADRB3 Trp64Arg polymorphism

No associations were found between the *ADRB3* Trp64Arg polymorphism and weight loss during the six month programme in the current study. A total of 13 other published studies could be traced that also investigated the association between the *ADRB3* Trp64Arg polymorphism and weight loss outcome. Three studies that included Caucasian populations showed no association between this polymorphism and weight loss after two 12-month interventions (Tchernof *et al*. 2000, Rawson *et al*. 2002) and one three-month weight loss intervention (De Luis *et al*. 2007). Another study reported that subjects who were carriers of the mutant *ADRB3* Arg64 allele and the mutant *UCP1* A/G allele were not able to maintain their weight loss during the 40-week weight maintenance phase after the 12-week weight loss phase of the programme (Fogelholm *et al*. 1998). Four of the seven weight loss interventions carried out in Japanese showed that wild-type Trp64Trp homozygotes experienced a more pronounced weight loss after following low energy diets and exercise recommendations (Kogure *et al*. 1998, Yoshida *et al*. 1995, Sakane *et al*. 1997b) or using a weight loss drug (Mazindol) (Shimizu *et al*. 2007) for three months. No associations with weight loss outcome were reported in the other three studies in Japanese (Nakamura *et al*. 2000, Shiwaku *et al*. 2003, Kuriyama *et al*. 2008), although one study did indicate that wild-type Trp64Trp homozygotes experienced decreases in visceral fat area and visceral-to-subcutaneous fat ratio (Nakamura *et al*. 2000). In Koreans no association between the *ADRB3* Trp64Arg polymorphism and weight loss outcome was found, but Trp64Trp homozygotes experienced greater decreases in total, visceral and subcutaneous fat areas (Kim *et al*. 2003). Finally, Trp64Trp homozygous Chinese obese children benefited more from a three-month intervention aiming at decreasing their fat and cholesterol intake to limit weight gain (Xinli *et al*. 2001). The improvements reported for other obesity related phenotype indicators in Trp64Trp homozygotes also raise

the question whether BMI is a sensitive marker for detecting changes in adiposity following weight loss interventions. The disparate findings that were reported for the Japanese subjects could be explained by the fact that the four studies finding an association only included obese (BMI≥30) subjects, while the three studies finding no associations included subjects with BMIs as low as 21.

Our finding of no association between the ARDB3 Trp64Arg polymorphism and weight loss outcome is thus supported by the other work done in Caucasians. However, the same is not true for the work done in Asian populations, where some associations have been reported.

ADRB2 Arg16Gly polymorphism

It was evident that participants with the mutant Gly16Gly genotype of the *ADRB2* Arg16Gly polymorphism in our study lost significantly less weight during the first month of the programme than the heterozygotes or wild-type homozygotes, although this association disappeared subsequently. When considering the gene-gene interactions it was also found that subjects with a mutant Gly16-allele of the *ADRB2* Arg16Gly polymorphism and a mutant C-allele of the *INSIG2* rs7566605 polymorphism lost significantly less weight over the six month intervention period. This finding does support the possibility that the Gly16-allele of *ADRB2* may be resistant to weight loss on a conservative weight loss programme when combined with a mutant C-allele of the *INSIG2* rs7566605 polymorphism.

Only one report could be traced that investigated the effect of this polymorphism on weight loss outcomes (Masuo *et al*. 2005). It can be argued that the result of the latter study is in agreement with our results as these authors reported a higher frequency of the Gly16-allele in overweight Japanese men who were resistant to weight loss during a 24 month weight loss programme compared to the men who lost weight. The results of two exercise interventions consisting of cycling sessions on ergometers three times a week for 20 weeks may also support our findings (Garenc *et al*. 2003, Ukkola *et al*. 2003). It was found that Black Arg16-allele carriers experienced greater decreases in fat mass and abdominal total fat area (Ukkola *et al*. 2003), while Caucasian white Arg16Arg homozygous females experienced greater reductions in BMI, fat mass and body fat percentage following the 20-week exercise programme (Garenc *et al*. 2003). It can be argued that the effect of the Arg16Gly polymorphism on the physiological function of ADRB2 also supports our findings. The *ADRB2* Gly16-allele has been associated with a more pronounced decrease in *ADRB2* expression in adipose tissue due to enhanced down-regulation after prolonged agonist stimulation (Green *et al*. 1994, Green *et al*. 1995). This may consequently result in reduced receptor function and lower efficiency of lipolysis stimulation (Ellsworth *et al*. 2002), which may explain lower weight loss rates over time.

Thus, although no association was evident between this polymorphism and weight loss over the total six months period it is argued that the association found for the first month is supported by our gene-gene interaction analysis, existing intervention evidence as well as the reported physiology of the mutant *ADRB2*. Further investigation of the association between this polymorphism and weight loss outcomes is therefore deemed necessary.

GNB3 C825T polymorphism

No associations were found between the *GNB3* C825T polymorphism and weight loss during the six month intervention period in the current study. This is in line with the results reported by Potoczna *et al*. (2004) who also found no association between this polymorphism and weight loss over a period of three years following bariatric surgery in Caucasian subjects. However, in contrast to these results Hauner *et al*. (2003) reported that Caucasian T-allele carriers benefited from a 54-week conservative weight loss intervention without pharmacotherapy to lose weight, while CC homozygotes benefited from the same conservative weight loss intervention if a weight loss drug (Sibutramine) was added. The latter study suggests that wild-type CC homozygotes will probably not lose weight on a programme consisting of a low energy, low fat diet; physical activity and behaviour modification recommendations only, and that they may need additional pharmacotherapy to achieve weight loss. However, the decreased GNB3 protein content found in subcutaneous adipocytes of TT homozygous Caucasian subjects compared to the wild-type CC subjects, with associated decreases in catecholamine induced lipolysis in TT subjects (Rydén *et al*. 2002) or T-allele carriers (Hauner *et al*. 2002), refutes the findings of Hauner *et al*. (2003). It can thus be argued that the findings of Hauner *et al*. (2002) and Rydén *et al*. (2002) suggest that CC subjects will respond better to a negative energy balance induced either by an increase in physical activity or a decrease in energy intake or a combination of the two because normal levels of lipolysis can occur in these subjects.

In the current study it was also found that a decrease in depressive symptoms in the CC subjects over four months resulted in a reduction in BMI that was not observed in the other genotype groups. The association between depression or response to anti-depression treatment and *GNB3* has been investigated by others (Lopez-Leon *et al*. 2008, Exton *et al*. 2003, Lee *et al*. 2004, Cao *et al*. 2007, Lin *et al*. 2001, Kunugi *et al*. 2002, Kang *et al*. 2007, Kato *et al*. 2008, Wilkie *et al*. 2007, Lin *et al*. 2009). However, these studies did not include weight loss outcome variables and therefore can not be used to elucidate the effect of the association between the CC genotype and depression on BMI in our study.

It is evident from the above discussion that the association between the *GNB3* C825T polymorphism and weight loss outcome is still equivocal and further investigation is necessary. The novel finding from this research that CC subjects may experience greater weight losses if an improvement in depressive symptoms occurs, warrants further investigation.

5.3 Conclusions

The weight loss programme used in this research was effective in inducing significant weight loss over a period of six months in overweight/obese subjects included in the study. The subjects also experienced significant improvements in specific lifestyle behaviours and psychological health indicators that can be attributed to the strategies included in the weight loss programme.

The primary null hypothesis, namely that there is no association between genotype and weight loss outcome, could not be rejected for the *FABP2* Ala54Thr, *INSIG2* rs7566605, *FTO* rs1421085, *ADRB3* Trp64Arg and *GNB3* C825T polymorphisms (Table 5.9).

However, the primary hypothesis was rejected for the *FTO* rs17817449 and *ADRB2* Arg16Gly polymorphisms as well as the gene-gene interaction between the *INSIG2* rs7566605 and *ADRB2* Arg16Gly polymorphisms, as the following was found (Table 5.9):

- The wild-type TT homozygotes of the *FTO* rs17817449 polymorphism lost significantly more weight during the first two months of the program compared to the mutant allele carriers. This is a novel finding.
- The wild-type Arg16Arg homozygotes of the *ADRB2* Arg16Gly polymorphism lost significantly more weight during the first month of the program compared to the mutant allele carriers. This finding is supported by one other intervention study.
- Subjects with a mutant C-allele of the *INSIG2* rs7566605 polymorphism and a mutant Gly16-allele of the *ADRB2* Arg16Gly polymorphism lost significantly less weight over the six month intervention period. This is a novel finding.

The secondary/exploratory hypotheses that were rejected are also depicted in Table 5.9. These were all novel findings and thus need further investigation for confirmation.

Table 5.9: A summary of findings from this study.

Findings from this study	**Evidence: Novel/ supported**
***FABP2* Ala54Thr**	
❑ Did not influence weight loss	❑ Supported
❑ Mutant Thr54Thr: improved general psychological well-being (GHQ) = ↓ weight during 6 months	❑ Novel

Table 5.9: *(continued)*

Findings from this study	Evidence: Novel/ supported
***INSIG2* rs7566605**	
❑ Did not influence weight loss on own	❑ Support conflicting, most point to C-allele = resistant to weight loss
❑ Haplotype: mutant C-allele of *INSIG2* & Gly16-allele of *ADRB2* = ↓ weight loss during 6 months	
***FTO* rs1421085**	
❑ Did not influence weight loss	❑ Novel
❑ Wild-type TT: improved emotional disinhibition = more pronounced ↓ in weight during 6 month period.	❑ Novel
❑ Changes in physical activity at work and eating behaviours e.g. disinhibition, habitual disinhibition and avoidance of fattening food (dietary restraint) influence weight loss during 6 months period	❑ Novel
***FTO* rs17817449**	
❑ Wild-type TT: ↑ weight loss during first 2 months of programme.	❑ Novel
***ADRB3* Trp64Arg:**	
❑ Did not influence weight loss	❑ Supported by research in Caucasians, may influence weight loss in Asians.
***ADRB2* Arg16Gly**	
❑ Wild-type Arg16Arg: ↑ weight losses during first month.	❑ Supported by existing evidence
❑ Haplotype: mutant C-allele of *INSIG2* & Gly16-allele of *ADRB2* = ↓ weight loss during 6 months	
***GNB3* C825T:**	
❑ Did not influence weight loss	❑ Equivocal support
❑ Wild-type CC: ↓ in depressive symptoms = ↓ in weight during 6 months period.	❑ Novel

Bearing in mind the limitations of this study (see Chapter 6, p250-251), it can be concluded that the *FTO* rs17817449 and *ADRB2* Arg16Gly polymorphisms and the interaction between the *INSIG2* rs7566605 and *ADRB2* Arg16Gly polymorphisms may influence weight loss outcomes in Caucasians. Furthermore, interactions between the *FTO* rs1421085 polymorphism and changes in emotional disinhibition, the *FABP2* Ala54Thr polymorphism and changes in general psychological well-being as well as the *GNB3* C825T polymorphism and changes in depressive symptoms may also influence weight loss.

Chapter 6

Final conclusions and recommendations

6.1 Integrated discussion

This chapter firstly focuses on perspectives on personalization of dietary intake and other health and lifestyle recommendations based on genetic screening for improved weight management and health. Secondly, perspectives on the level of evidence required to consider genetic screening in evidence based practice in dietetics is discussed. The question whether the associations found between genotypes of the seven polymorphisms included in this study and BMI, health and lifestyle indicators as well as weight loss outcomes, are robust enough for inclusion in genetic screening for personalization of weight management guidelines is also argued by integrating the findings from the cross-sectional and intervention studies as well as published evidence. Finally, conclusions and recommendations are provided.

It has been proposed that nutritional genomics and more specifically, genetic screening, will ensure that dietitians and other health professionals will ultimately move away from generalized nutrition recommendations to prescribing more genotype-based personalized diets and other lifestyle recommendations to prevent/treat chronic diseases (DeBusk *et al*. 2005, Trujillo *et al*. 2006, Boehl 2007). The potential benefits of such recommendations include early identification and prevention, cost-effective and targeted interventions (DeBusk *et al*. 2005, Joost *et al*. 2007, Stover & Caudill 2008) and increased motivation of individuals to follow the specified guidelines (Joost *et al*. 2007).

Although very promising, the negative implications of genetic screening also need to be considered. Joost *et al*. (2007) maintain that one of the main risks of personalized genotype-based nutrition is that recommendations that are not supported by sufficient robust evidence may be made to vulnerable individuals. Furthermore, the long-term safety of recommendations that are made may not have been tested in long-term follow-up studies (Joost *et al*. 2007). The issues of confidentiality of results, access to certain insurance products and discrimination in the workplace have also been raised (Gable *et al*. 2007). However, Castle and Ries (2007) maintain that specific strategies can be employed to overcome the rising ethical and legal issues and that this should not pose a problem.

Sanderson and Michie (2007) point out that it is also important to take into account the possible psychological and behavioural impact of genetic screening. While genetic screening may lead to less healthy behaviour in individuals who are not genetically susceptible to develop chronic diseases such as obesity (Frosch *et al*. 2005), preliminary research also indicates increased motivation to change behaviour and improvements in obese subjects' beliefs that changing behaviour will reduce disease risk (Harvey-Berino *et al*. 2001). Furthermore, Arkadianos *et al*. (2007) indicated that genotype tailored diets resulted in better compliance and weight loss over a one year period in screened individuals compared to the control group.

Genetic screening has been used effectively in the past to screen for relatively uncommon monogenic disorders, but has now become increasingly available on the market for chronic disease prevention/management. In many countries, including South Africa, companies have started marketing genetic screening for chronic disease prevention/treatment through health care providers or directly to the public, even in the form of home-testing kits (DeBusk *et al*. 2005, www.dnadiet.co.za). Research shows that there is a high level of interest among the public in genetic testing for multifactorial diseases such as cancer and heart disease risk (Graham *et al*. 1998, Sanderson *et al*. 2004, Sanderson & Wardle 2008). This is facilitated by the lay media's promotion of new concepts and research findings to be on the frontline of reporting (Boehl 2007). Such direct marketing of tests for which sufficient research to support the claims is not yet available, may be harmful to the public, who may be unable to make informed choices in this regard (Gollust *et al*. 2002, Sanderson *et al*. 2004). Marketing of genetic screening tests and consequent personalization of dietary recommendations for obesity prevention/treatment should only take place if it is evidenced-based and has clinical utility (Evans *et al*. 2001, Gray & Gray 2002). To ensure evidence based practice, research should thus provide evidence that the recommended dietary guidelines or other lifestyle recommendations for a genotype group are linked to a robust increase or decrease in disease risk. Furthermore, repeatable evidence from genotype-based interventions should be available to prove that the suggested genotype specific recommendations reduce the risk factors for a specific disease, the disease itself or will maximize treatment outcome (Evans *et al*. 2001, Joost *et al* .2007).

In 2005 Kaput *et al*. pointed out that no internationally uniform standards existed for the design of gene-diet-disease research (Kaput *et al*. 2005) and for the interpretation of the results derived from current and future research (Kaput *et al*. 2005, Joost *et al*. 2007). In 2007, Joost *et al*. proposed the following guidelines for research design: Firstly, the sample should be selected in such a way that it includes equal numbers of subjects carrying the wild-type, heterozygous and mutant homozygous genotypes. Secondly, each genetic group should follow each specific lifestyle intervention, which should be rotated across the interventions (Joost *et al*. 2007). However, the practicality and effectiveness of the latter recommendation for research on weight loss outcomes is questionable bearing in mind that obese individuals usually lose weight in the beginning of a weight reduction programme, but often reach a weight loss plateau after time as the body defends its own weight set-point (Peters 2006). Guidelines for interpretation of results suggested by Joost *et al*. (2007) are as follow: Firstly, if there is a small variation in the results of a study, thus most individuals in a genotype group lost weight, personalized recommendations could probably be made. However, if there is a large variation in results, too many unknown factors (genetic or environmental) may have played a role and personalized recommendations may not be warranted (Joost *et al*. 2007).

At this point in time evidence regarding the association between genotype, obesity and weight loss outcomes seems to be equivocal for most investigated genes (Rankinen *et al*. 2006, Hainer *et al*. 2008). It is

argued that this situation is due to a lack of homogeneity in the different study samples regarding ethnicity, gender, age differences, differences in adiposity levels, absence or presence of concomitant disorders (Hainer *et al*. 2008, Tiwari *et al*. 2008). The results can also be affected by the use of different study designs as well as power estimates, population stratification, genome wide association vs. candidate/pathway approach, Hardy Weinberg Equilibrium, genotyping success and errors, frequency of studied genetic variants (Lewis *et al*. 2002, Andersson *et al*. 2009) and the different statistical tests and models used across studies (Jakobsdottir *et al*. 2009). Furthermore, contradictory results can also be attributed to false-positive findings as multiple comparisons are often used in genetic association studies, which increase the likelihood of a significant association occurring once in every 20 tests (Andersson *et al*. 2009). Lastly, the fact that the sum of a number of diet-gene-risk factor interactions that vary amongst individuals are probably involved in the beneficial effects associated with a particular lifestyle intervention (Joost *et al*. 2007), compound the issue.

With this research we set out to provide further evidence on the associations between genotype and BMI (primary hypothesis), health and lifestyle indicators (secondary and exploratory hypotheses) as well as weight loss outcomes (primary hypothesis) for the seven polymorphisms included in this study. In the following sections the results of the cross-sectional and intervention studies are integrated with relevant published evidence to provide the robustness of the evidence for inclusion in genetic screening.

The FABP2 Ala54Thr polymorphism

The fact that the current study and four other previously reported studies found no association between the *FABP2* Ala54Thr polymorphism and weight loss outcome after following a conservative weight loss diet (current study, De Luis *et al*. 2006, De Luis *et al*. 2008a, Takakura *et al*. 2005), bariatric surgery (De Luis *et al*. 2008b) or a low carbohydrate diet (De Luis *et al*. 2008a) may indicate that this polymorphism is not important to be considered for personalized weight loss recommendations based on genotype. However, published evidence does point to the possibility that the *FABP2* Ala54Thr polymorphism influences the outcome of other health and obesity phenotype indicators following weight loss interventions, favouring mostly the Ala54Ala subjects, although results are still equivocal. The modelled results from our cross-sectional sample support the notion that weight loss could be beneficial for the wild-type Ala54Ala subjects in terms of decreasing their TG levels, which was not found for the mutant Thr54-allele carriers. This finding is supported by De Luis *et al*. (2008a) but no association was found by De Luis *et al*. (2008b).

As existing evidence also indicates that Thr54-allele carriers may have a higher fat absorption, specifically for SFA and PUFA (Richieri *et al*. 1994, Baier *et al*. 1995, Marín *et al*. 2005), it is proposed that specific macronutrient modifications may be necessary for Thr54-allele carriers in a weight loss programme. The literature points to the possibility that Thr54-allele carriers will benefit from a low fat, SFA and PUFA diet or

a higher MUFA intake. The fact that Thr54Thr homozygotes in our cross-sectional study were found to have a higher frequency of intake of take-away foods that are typically high in fat, SFA and PUFA emphasises the need for such subjects to focus on healthier fat choices. Further investigation into the response of each *FABP2* genotype to diets with different fatty acid contents therefore seems justified. However, at this stage it must be borne in mind that low fat recommendations would benefit all individuals regardless of genotype, although it may benefit the Thr54-allele carriers most.

The novel findings in this research point to the possibility that Thr54Thr subjects have a higher self-esteem and that they experienced the most pronounced weight loss over the six months intervention period in response to improvements in psychological health. To eliminate the possibility that these findings are false positives further investigation into the physiological explanation thereof as well as the replication of our findings in independent weight loss intervention and case-control studies, is necessary.

The INSIG rs7566605 polymorphism

The *INSIG2* rs7566605 polymorphism did not influence weight loss outcome in the current study. However, with gene-gene interaction analysis it was found that subjects with a mutant C-allele of the *INSIG2* rs7566605 polymorphism and a mutant Gly16-allele of the *ADRB2* Arg16Gly polymorphism lost significantly less weight over the six month intervention period. This is a novel finding that indicates that the two polymorphisms have a combined effect on weight loss. The evidence from other intervention studies regarding the association between the *INSIG2* rs7566605 polymorphism and weight loss outcome is equivocal studies (Reinehr *et al*. 2008, Franks *et al*. 2008, Orkunoglu-Suer *et al*. 2008, Reinehr *et al*. 2009). The majority of these studies point to the possibility that C-allele carriers are resistant to weight loss, while GG homozygotes experience weight loss on a conservative weight loss programme. It can also be argued that the findings from our cross-sectional sample support this possibility, as GG homozygotes experienced the most pronounced modelled decrease in BMI in response to an increase in leisure-time physical activity. Furthermore, although we found that C-allele carriers in the cross-sectional sample had higher leisure-time physical activity levels, there were no differences in the mean BMI between the genotype groups, supporting the notion that C-allele carriers may be resistant to weight loss when increasing physical activity levels. The possibility that C-allele carriers do not respond to conservative weight loss treatment may also explain our results from the cross-sectional sample, indicating that the mutant CC homozygotes had a higher risk to develop MetS. It is also argued that these results are supported by previous reports indicating a higher BMI in CC subjects (Herbert *et al*. 2006, Lyon *et al* 2007, Rosskopf *et al*. 2007, Liu *et al*. 2008, Andreasen *et al*. 2008), as well as the possible effect of a variant INSIG protein and associated changes in the physiological function thereof (Gong *et al*. 2006). However it must be borne in mind that the *INSIG2* rs7566605 polymorphism is located 10 kb upstream of the transcription start site of the *INSIG2*

gene (Herbert *et al*. 2006) and it is still a question whether this variant itself or maybe another variant in strong LD leads to the expression of a variant INSIG2 protein.

The novel findings from the cross-sectional study that the mutant CC homozygotes had a lower attitude to self-regulation score and that they are likely to experience a modelled increase in BMI as their situational disinhibition score increases, warrant further investigation. It is argued that the proposed changes in the physiological functioning of a variant INSIG2 protein may result in an inability to block lipogenesis, which could be to the disadvantage of CC subjects, as the latter were shown to be more likely to exhibit poor eating behaviours, which may result in increased fat and energy intakes, consequently resulting in a higher BMI, MetS risk as well as resistance to weight loss interventions.

The FTO polymorphisms

Several novel findings for the *FTO* polymorphisms that warrant further investigation were found in the current study. These findings include the following:

- The wild-type TT homozygotes of the *FTO* rs17817449 polymorphism experienced a greater weight loss over the first two months of the programme. This can also be interpreted as a resistance to weight loss on a conservative weight loss programme in the mutant allele carriers, at least during the first two months of treatment. As this is a novel finding, which was only significant for the genotype model and no association between weight loss outcome and another *FTO* polymorphism (in strong LD with the *FTO* rs17817449 polymorphism) has been reported by two other studies (Müller *et al*. 2008, Franks *et al*. 2008) the possibility that this may be a false positive needs to be considered.
- An improvement in emotional disinhibition resulted in the most pronounced BMI loss over a six month period in the wild-type TT subjects of the *FTO* rs1421085 polymorphism. This finding was supported by the modelled cross-sectional data indicating that a modelled improvement of the attitude to self-regulation was associated with the most pronounced modelled decrease in BMI of the wild-type TT subjects. On the other hand a modelled increase in rigid control was associated with the most pronounced increase in BMI of the mutant CC homozygotes of the *FTO* rs1421085 polymorphism. Genotype interaction effects from the intervention study between the *FTO* rs1421085 polymorphism and changes in eating behaviours (including disinhibition, habitual disinhibition and avoidance of fattening foods) on BMI loss over a six months period are also evident. Furthermore, the results from the cross-sectional sample also showed various significant associations between the *FTO* polymorphisms and eating behaviour including the following: the mutant allele of either the *FTO* rs1421085 or rs17817449 polymorphism were associated with a higher intake of high fat foods as well as with poorer eating behaviours as reflected in higher scores for perceived hunger, internal locus for hunger, habitual and emotional susceptibility to disinhibition and rigid control of dietary restraint. These associations are supported by the associations found by others between food intake or eating

behaviour and *FTO* polymorphisms (Bray *et al*. 2004a, Rosmond *et al*. 2004, Malik *et al*. 2006, Gibson 2008, Wolff & Dansinger 2008) that are in strong LD with the two *FTO* polymorphisms investigated in this research. It is thus evident that the mutant alleles of the two *FTO* polymorphisms included in this study may predispose individuals to having poorer eating behaviours, while an improvement in eating behaviour may specifically benefit the wild-type homozygotes to lose weight. Although the function of FTO and its role in obesity development still need to be elucidated, it has been proposed that *FTO's* expression in the hypothalamus (Dina *et al*. 2007b, Frayling *et al*. 2007, Gerken *et al*. 2007) is nutritionally regulated (Stratigopoulos *et al*. 2008) and that FTO is possibly involved in energy homeostasis by influencing energy expenditure (Fischer *et al*. 2009) as well as energy intake and eating behaviour (Speakman *et al*. 2008, Wardle *et al*. 2009)

- The findings from the cross-sectional study showed that the T-T haplotype of the two *FTO* polymorphisms experienced a modelled decrease in BMI in response to an increase in the sport index score. This finding may be supported by that fact that a significant genotype interaction effect was also found between this polymorphism and the work index score on BMI losses over the six month intervention period. The proposed physiological function for *FTO* supports the involvement thereof in energy expenditure.
- Further novel findings from the cross-sectional study indicate that a modelled increase in BMI was associated with the most pronounced decrease in HDL levels of the mutant homozygotes of both *FTO* polymorphisms, which was confirmed with haplotype analysis. The physiological context for these findings still needs to be elucidated.
- The results from the cross-sectional sample also indicate that the mutant GG subjects of the *FTO* rs17817449 polymorphism may have more depressive symptoms while a modelled improvement in general psychological well-being was associated with the most pronounced decrease in BMI in the wild-type TT subjects of the *FTO* rs1421085 polymorphism. The physiological context for these novel findings still needs to be elucidated.

The ADRB3 Trp64Arg polymorphism

This polymorphism did not influence weight loss outcome in the current study, which is in line with the results of three published weight loss interventions performed in Caucasian populations (Tchernof *et al*. 2000, Rawson *et al*. 2002, De Luis *et al*. 2007). In Asian samples, seven of the nine published weight loss interventions found that the wild-type Trp64Trp homozygotes experienced greater reductions in weight or fat area, while the Arg64-allele carriers were resistant to weight loss (Kogure *et al*. 1998, Yoshida *et al*. 1995, Sakane *et al*. 1997b, Nakamura *et al*. 2000, Xinli *et al*. 2001, Kim *et al*. 2003, Shimizu *et al*. 2007). It can be argued that the latter findings are supported by the latest meta-analysis that included 97 studies, which found that the Arg64-allele predisposes Asians to having a higher BMI or obesity prevalence, but no association was found for Caucasians (Kurokawa *et al*. 2008). It must be noted that 90% of the current

sample were wild-type Trp64Trp homozygotes, 10% were heterozygotes and none were mutant homozygotes, leading to a mutant allele frequency of 5%. This is in line with the genotype and allele frequencies reported for other Caucasian populations, while it is a fact that Asian populations have a higher mutant allele frequency of ~20%. Yang *et al*. (2005) proposed that fewer genes can be included in a personalized screen for polygenic diseases if the frequencies of predisposing genotypes are common in the specific population. They recommend that "only around 20 genes are usually needed to explain 50% of the burden of a disease in the population if the predisposing genotypes are common (>25%), even if the individual risk ratios are relatively small (i.e. increasing the risk by only 20-50%)". In comparison to all the other polymorphisms included in this research, the *ADRB3* had the lowest mutant allele frequency, while those for the other polymorphisms were definitely more "common" (34 to 58%). We propose that this polymorphism could thus be included in an investigation of weight loss outcomes for the South African Asian/Indian race group as well as Black South Africans as a mutant allele frequency of 28% has previously been reported for the latter group. However, due to the fact that no associations in the cross-sectional as well as the intervention sample with the *ADRB3* Trp64Arg polymorphism were found and the fact that a low mutant allele frequency was found, it is suggested that *ADRB3* may not be an important gene to be considered for personalization of weight loss guidelines for Caucasian South Africans.

The ADRB2 Arg16Gly polymorphism

In the current study it was found that the wild-type Arg16Arg homozygotes of the *ADRB2* Arg16Gly polymorphism experienced greater weight losses, especially during the first month of treatment. This finding is supported by existing evidence from one weight loss (Masuo *et al*. 2005) and two physical activity interventions (Garenc *et al*. 2003, Ukkola *et al*. 2003) as well as the changes in the physiological function of ADRB2 that occur as a result of the mutant allele (Green *et al*. 1994, Green *et al*. 1995, Ellsworth *et al*. 2002). The findings from the gene-gene interaction analysis further indicate that subjects with the mutant Gly16-allele of *ADRB2* and the mutant C-allele of *INSIG2* lost significantly less weight over the six month intervention period.

It can further be argued that the Gly16Gly homozygotes may be more inclined to gain weight during adolescence and young adulthood. This is based on the premises that more Arg16Arg homozygotes thought they were overweight during childhood, an association that was not evident any more during adolescence, young adulthood and at the time of the study. As the literature in this regard is still equivocal, further research is necessary to confirm this association.

The novel finding from the cross-sectional sample that Arg16Arg homozygotes experienced the most pronounced decrease in BMI in a response to an improvement of their flexible control of dietary restraint

needs to be confirmed as no association study or physiological context link has been identified to support the possibility that it is a true association.

The GNB3 C825T polymorphism

No association between the *GNB3* C825T polymorphism and weight loss was found in the current study, which is in line with the finding of one published weight loss intervention (Potoczna *et al*. 2004). However, the results from another published weight loss intervention reported increased weight loss in T-allele carriers on a conservative weight loss programme, with CC subjects responding if Sibutramine was added (Hauner *et al*. 2003). The latter finding can not be explained within the context of the known physiological function of the variant GNB3 protein in adipocytes. It is also in contrast with the novel findings from the cross-sectional sample that suggest that CC subjects may respond more effectively to a conservative weight loss diet that places emphasis on increasing leisure-time physical activity as well as decreasing intake of high fat foods, energy-dense snacks and drinks. The lower GNB3 protein levels in adipocytes as well as the decreased stimulation of lipolysis in mutant allele carriers (Hauner *et al*. 2002, Ryden *et al*. 2002) may explain why the CC subjects respond better to physical activity or decreased energy-dense snacks to lose weight, as normal lipolysis probably occurs in CC subjects.

Normal lipolysis stimulation may also explain the more favourable MetS profile found in the CC subjects. The cross-sectional results indicate that mutant TT homozygotes had higher triglyceride and glucose levels, a higher number of traits that met the diagnostic cut-offs for MetS and a higher number who were diagnosed with MetS. These findings are in line with other published reports in Caucasian populations (Poch *et al*. 2002, Brand *et al*. 2003, Wascher *et al*. 2003, Andersen *et al*. 2006) and can be explained by the possible.

Another novel finding from the intervention sample indicates that CC subjects may experience greater weight losses over a six month period if an improvement in depressive symptoms occurs. The novel finding from the cross-sectional sample indicates that CC subjects may have a lower self-esteem. The finding from the cross-sectional sample that CC subjects in our study had more depressive symptoms is not in line with previous reports that indicate either no association or an association between the T-allele and depression (Exton *et al*. 2003, Lee *et al*. 2004, Cao *et al*. 2007, Lopez-Leon *et al*. 2008). As most of this research involved Asian populations, more research is necessary to elucidate the association between depressive symptoms and this polymorphism in Caucasian populations.

6.2 Final conclusions and recommendations

Bearing in mind the limitations of the current study, the following conclusions can be made regarding the primary hypotheses of the cross-sectional and intervention studies:

- There is no association between the BMI of overweight/obese subjects and all the polymorphisms investigated. These results were confirmed by meta-analyses for the *INSIG2* rs7566605, *ADRB3* Trp64Arg, *ADRB2* Arg16Gly and *GNB3* C825T polymorphisms. For the *FTO* rs1421085 and rs17817449 polymorphisms current literature consistently link the mutant genotype with a higher BMI. The published results for the *FABP2* Ala54Thr are still equivocal.
- There is no association between weight loss outcome and the *FABP2* Ala54Thr and *ADRB3* Trp64Arg polymorphisms, which is supported by published findings in Caucasian populations.
- There is no association between weight loss outcome and the *GNB3* C825T polymorphism. Literature in this regard is still equivocal and thus warrants further investigation.
- The wild-type TT homozygotes of the *FTO* rs17817449 polymorphism lost significantly more weight during the first two months of the program compared to the mutant allele carriers. As this is a novel finding, which was only significant for the genotype model and no association between weight loss outcome and another *FTO* polymorphism has been reported by two other studies the possibility that this may be a false positive needs to be considered.
- The wild-type Arg16Arg homozygotes of the *ADRB2* Arg16Gly polymorphism lost significantly more weight during the first month of the program compared to the mutant allele carriers. This finding is supported by one other intervention study.
- Subjects with a mutant C-allele of the *INSIG2* rs7566605 polymorphism and a mutant Gly16-allele of the *ADRB2* Arg16Gly polymorphism lost significantly less weight over the six month intervention period. This is a novel finding that warrants further investigation as an association with the individual genes and weight loss has been indicated as well as a physiological context link supports this possibility.

The secondary and exploratory hypotheses that were formulated for the cross-sectional and intervention studies were rejected in a number of instances. The main findings that stood out in terms of support from the literature and a plausible physiological explanation include:

- The mutant TT homozygotes of the *GNB3* C825T polymorphism may have a higher risk for the development of MetS and MetS traits.
- The mutant allele carriers of the *FTO* rs1421085 and rs17817449 polymorphisms may have poorer eating behaviours while an improvement in eating behaviour may specifically benefit the wild-type homozygotes to lose weight.

It is important to consider that the cross-sectional results are limited by the fact that a normal weight control group was not included. It is therefore necessary to emphasize that the cross-sectional results pertain to overweight/obese individuals only and should be confirmed in further case-control and intervention studies for broader application.

It is also important to bear in mind that BMI (the obesity phenotype indicator used in this study) seems to have limitations as a general indicator of adiposity. It is thus possible that using more specific phenotype indicators of adiposity e.g. subcutaneous/ abdominal fat area, body fat % etc. may have resulted in different outcomes.

As most obesity related polymorphisms are expected to have a small effect on obesity development or weight loss outcome, the small sample size as well as the fact that only an average of 80% genotyping success rate was achieved in the current study restricted statistical power of analyses. The high attrition rate in the intervention study compounded the problem. From the literature review it is clear that only a limited number of studies have been performed on the association between genotype and weight loss outcomes. From our experience it is clear that this could be ascribed to the complexity and time-consuming aspects of weight loss interventions, which may also explain the small sample sizes included in many of these studies. However, investigations in larger samples that ensure appropriate statistical power are essential to ensure that evidence re the investigated associations is robust before application in clinical practice is warranted.

The fact that no follow-up blood values were collected may be seen as a limitation of the intervention study. However, the decision to exclude MetS diagnosis at follow-up was made because the aim of the intervention study was to assess the association between genotype and weight loss outcomes, not associated physical health indicators. The same can be said for the fact that dietary intake was only assessed at baseline and not at follow-up. This decision was based on the need to decrease respondent burden. Past experiences in this regard guided this decision as the completion of lengthy questionnaires was stated as a reason for not returning to follow-up measurements (Cilliers 2004). For the same reason the questionnaires on the other psychological health and lifestyle variables were only administered at 16 week follow-up and not again at 24-weeks. It was also argued that the time-period between the 16 and 24 weeks was too short for measurable changes to occur in these variables. A further drop-out was also expected at 24 weeks compared to 16 weeks, which would have resulted in an even smaller sample size.

As many variables were self-reported (in this research: physical activity, dietary intake, eating behaviour and psychological health), it needs to be mentioned that self-reported data is always limited in that it has to be assured that the reported information is the truth.

The use of multiple comparisons as performed in this thesis may be seen as a limitation as it is difficult to distinguish between false positive and true associations. Multiple comparisons are however recognized as a common phenomenon in genetic association studies (Andersson *et al*. 2009). Perneger (1998) recommends that it is important to consider the results from all association tests and that each significant p-value should rather be interpreted with caution, taking into account the plausibility of the finding. In the current study, the replication of previously reported results as well as functional explanation of the association between the polymorphism and phenotype (Andersson *et al*. 2009) where considered as factors that strengthens the possibility that the specific finding may be a true association. The novel associations found in the current study should thus be viewed as "hypotheses generating" findings that should be replicated and further investigated to establish the functional plausibility and clinical value for implementation as part of weight management interventions.

Finally, although the *FTO* rs17817449 and *ADRB2* Arg16Gly polymorphisms as well as the gene-gene interaction between the *ADRB2* Arg16Gly and *INSIG2* rs7566605 polymorphisms influenced weight loss over the short-term in the current study, it would be premature to conclude that these polymorphisms can be included as part of an evidence-based personalized weight loss recommendation program. Within this context it must also be borne in mind that genotype only explains one component of the phenotypic expression of multifactorial diseases such as obesity or the response to weight loss treatment. Effective obesity prevention/treatment interventions should thus address the spectrum of causes of the condition that can include any combination of genetic, biochemical, psychological, physiological and environmental factors. Furthermore, with the known fact that multiple polymorphisms play a role in the development of complex diseases such as obesity, more research such as the current study is necessary, but with the inclusion of investigating more polymorphisms and larger samples. If affordable, this requires more extensive analyses techniques such as DNA chip analysis or exome or whole genome sequencing.

Based on the results and conclusions of this study it is recommended that the following associations should be viewed as priority in future research:

- The *GNB3* C825T polymorphism and physical health indicators (MetS/MetS traits).
- The *FTO* rs1421085 and rs17817449 polymorphisms and weight loss outcome and eating behaviours.
- The gene-gene interaction between the *INSIG2* rs7566605 and *ADRB2* Arg16Gly polymorphisms and weight management.

Chapter 7

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Electronic database information

DNA diet: www.dnadiet.co.za

UK quack watch: www.quackwatch.org

WHO 2010; global obesity database: http://apps.who.int/bmi/index.jsp

NCBI 2008, 2009: http://www.ncbi.nlm.nih.gov/gene?term

Statistica (Statsoft Inc. (2009) STAT data analysis software system), version 9.

Addenda

Addendum A: Screening questionnaire

Screening Questionnaire

Today's date | | | / | | | / | 2 | 0 | 0 | |
d d m m y y y y

Name ..... Surname: .....

Date of birth | | | / | | | / | 1 | 9 | | | Age: | | | years
d d m m y y y y

Weight | | , | | kg Height | | , | | | m BMI | | | , | |

Highest academic qualification achieved .....

What is your profession currently .....

Please answer yes or no to the following questions

Are you currently pregnant	1. Yes ☐	2. No ☐	3. Not applicable ☐
Are you currently breast feeding	1. Yes ☐	2. No ☐	3. Not applicable ☐
Have you ever been diagnosed with Anorexia	1. Yes ☐	2. No ☐	
Have you ever been diagnosed with Bulimia	1. Yes ☐	2. No ☐	
Have you ever been diagnosed with a Psychiatric illness	1. Yes ☐	2. No ☐	
If yes, specify:			
Do you have a history of drug or alcohol abuse	1. Yes ☐	2. No ☐	
Do you suffer from Graves disease	1. Yes ☐	2. No ☐	
Do you use weight reduction aids on a regular basis	1. Yes ☐	2. No ☐	

If yes, specify: Name(s) of aids: .....

How often using it: .....

Contact details of potential subject

Telephone (home) ( ) ..... Cell phone .....

Telephone (work) ( ) ..... E-mail .....

In which town/ city/ suburb do you live? Stellenbosch ☐ Somerset-West ☐ Strand/ G'bay ☐ Paarl ☐

Other, specify ☐ .....

In which town/ city/ suburb do you work? Stellenbosch ☐ Somerset-West ☐ Strand/ G'bay ☐ Paarl ☐

Other, specify ☐ .....

Addendum B: Consent form

PARTICIPANT INFORMATION AND INFORMED CONSENT FORM

TITLE OF RESEARCH PROJECT: Association between conventional (dietary, physical activity and behavioural) treatment outcome (weight loss) in overweight/obese adults and genotype.

REFERENCE NUMBER:

PRINCIPAL INVESTIGATOR: Dr Marjanne Senekal

ADDRESS: Department of Physiological Sciences
University of Stellenbosch
Private Bag X1, Matieland, 7602

CONTACT NUMBER: (021) 8083631

We would like to invite you to participate in a research study that involves DNA (genetic) analysis and possible long-term storage of blood or tissue specimens. Please take some time to read the information presented here which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part initially.

This research study has been approved by the ethics **Committee for Human Research at Stellenbosch University** and it will be conducted according to international and locally accepted ethical guidelines for research, namely the Declaration of Helsinki, Guidelines on Ethics for Medical and Genetic Research of the Medical Research Council of South Africa (MRC).

WHAT IS DNA ANALYSIS OR GENETIC RESEARCH?

Genetic material, also called DNA, is usually obtained from a small blood sample. Occasionally other tissues may be used. DNA consists of numerous genes, strung together in long strands and found in every cell in the human body. Genes determine who we are, what we look like and sometimes what kind of diseases we may be susceptible to. Worldwide research in this field is continuously discovering new information that may be of great benefit to future generations and also that may benefit people today, who suffer from particular diseases or conditions.

What does this particular research study involve?
The aim of this research is to look at the success (=weight loss) of treatment of overweight adults with a conventional weight reduction program (including dietary, physical activity and behavioural components) and their genotype. We also want to identify appropriate predictors of long-term success of such conventional treatment. This will help to improve the matching of patients with treatment and thus the eventual success with treatment. As part of this project genetic material (blood) will be collected and analysed for the presence of certain genetic alterations that have previously been studied in overweight adults. These genetic alterations are most probably not associated with any negative effect(s) when present on their own, which means that they will only have an effect when combined with other genetic alterations and/or specific lifestyle patterns.

Why have you been invited to participate?
You fit the criteria for this study and were therefore invited to participate.

What procedures will be involved in this research?
You will be requested to:

- Provide information about your medical history, weight history, family's weight history, eating behaviour, dietary intake, physical activity, mental health and brief socio-demographic information by completing questionnaires, which will take 30-45 minutes;
- Keep record of everything that you eat and drink for 3 days on dietary record forms that will be provided to you (at the beginning of the project and then every two weeks for a period of six months);
- Keep a simple daily record (tick of activities as you did it) of your physical activity for the entire six month period;
- Give consent for the drawing of blood (15ml, about 3 teaspoonfuls) for the determination of a fasting (after 12-14 hours fast) blood HDL, triglyceride and glucose levels and for DNA-analysis;
- To obtain written permission from your general practitioner to participate in this research on the provided form;
- Be available for the assessment of the following anthropometric measurements: weight, height, waist circumference, hip circumference and triceps skin fold;
- Place a piece of blotting paper (3 cm diameter), which is saturated with a chemical compound that can have a bitter taste for some, on your tongue so that your taste sensitivity can be tested;
- Follow an individualized weight loss program, including a group session every two weeks for a period of six months that will include dietary, exercise, behavioural and cognitive restructuring components.

Are there any risks involved in genetic research?

- You may experience minor pain or bruising at the site where blood is taken;
- You may feel some discomfort when tasting the piece of paper, which is saturated with a harmless chemical compound that may taste bitter for some;
- You may feel some discomfort during the assessment of the anthropometric measurements and during the assessment of resting energy expenditure;
- Some insurance companies may mistakenly assume that taking part in genetic research indicates a higher risk for disease. Thus no information about you or your family will be shared with such companies;

Are there any benefits to your taking part in this study and will you get told your results?
You will gain scientific knowledge about weight management, you will receive a published weight management manual and will lose weight if you follow the program. After all the results of all the tests from all the study participants are known, a study conclusion written in layman terms will be provided to you.

How long will your blood be stored and where will it be stored?
Your blood will be stored for a period of 5 years at the Department of Physiological Sciences, University of Stellenbosch.

If your blood is to be stored is there a chance that it will be used for other research?
Your blood and DNA will only be used for genetic research that is directly related to the treatment of overweight or obesity. Also if the researchers wish to use your stored blood for **additional research in this field** they will be required to apply for permission to do so from the Human Research Ethics Committee at Stellenbosch University.

If you do not wish your blood specimen to be stored after this research study is completed you will have an opportunity to request that it be discarded when you sign the consent form.

How will your confidentiality be protected?
Your identity will be kept confidential throughout and information will not be associated with your name. The research staff will use only a coded number, access will be limited to authorized scientists and any scientific publications, lectures or reports resulting from the study will not identify you by name.

Will you or the researchers benefit financially from this research?
You will not be paid to take part in this study and will not be compensated for travelling costs to attend the initial assessments and group sessions every two weeks.

In the unlikely event that the research leads to the development of a commercial application or patent you or your family will not receive any profits or royalties. However profits will be reinvested to supporting the cause of further research, which may bring benefits to your family or community in the future.

Declaration by participant

By signing below, I ……..…… agree to take part in a genetic research study entitled: Association between conventional (dietary, physical activity and behavioural) treatment outcome (weight loss) in overweight/obese adults and genotype.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is voluntary and I have not been pressurised to take part.

☐ I agree that my blood or tissue sample can be stored, but I can choose to request at any time that my stored sample be destroyed. I have the right to receive confirmation that my request has been carried out.

OR

☐ Please destroy my blood sample as soon as the current research project has been completed. **(Tick the option you choose**)

Signed at (*place*) .....…..…… on (*date*) ……..…….. 2006.

.....	.....
Signature of participant	Signature of witness

Declaration by investigator

I *(name)* …… declare that:

- I explained the information in this document to ……..…….....
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research as discussed above.
- I did/did not use a translator. (*If a translator is used then the translator must sign the declaration below).*

Signed at (*place*) .....…..……..….. on (*date*) ……...…….…….. 2006.

.....	.....
Signature of investigator	Signature of witness

Addendum C: Questionnaires

Sociodemographic & weight questionnaire

Date			/			/	2	0	0	7	Code				
	d	*d*		*m*	*m*		*y*	*y*	*y*	*y*					

Name & Surname

1. Gender
   1. Male ☐
   2. Female ☐

2. Marital status
   1. Unmarried ☐
   2. Married ☐
   3. Divorced ☐
   4. Separated ☐
   5. Widowed ☐
   6. Living together ☐

3. Home language
   1. Afrikaans ☐
   2. English ☐
   3. Zulu ☐
   4. Other, specify : ☐

   ….....

4. Highest level of education completed
   1. Matric ☐
   2. College certificate/ diploma ☐
   3. Technikon diploma/ degree ☐
   4. University degree ☐
   5. Other, specify : ☐

   .....

5. Do you live
   1. alone ☐
   2. with friends ☐
   3. with your husband/ wife/ partner ☐
   4. with your husband/ wife/ partner and children ☐
   5. With your parents ☐
   6. Other, please specify: ☐

   .....

6. In which of the following areas do you stay?
   1. Stellenbosch/ Somerset-West/ Strand/ Gordonsbay ☐
   2. Northern suburbs: Durbanville, Brackenvelle, Bellville etc. ☐
   3. Southern suburbs: ?? ☐
   4. Cape Town: city centre ☐
   5. Other, please specify: ☐

   .....

7. Would you describe your weight during most of your childhood years (ages 2 to 10) as:
   1. Underweight ☐
   2. Normal weight ☐
   3. Overweight ☐
   4. Obese ☐

8.	Would you describe your weight during most of your adolescent years (ages 11 to 19) as:	1. Underweight ☐ 2. Normal weight ☐ 3. Overweight ☐ 4. Obese ☐
9.	When you were between the ages of 20 to 25 years would you describe your weight as:	1. Underweight ☐ 2. Normal weight ☐ 3. Overweight ☐ 4. Obese ☐ 5. Not applicable ☐
10.	When you were between the ages of 25 to 30 years would you describe your weight as:	1. Underweight ☐ 2. Normal weight ☐ 3. Overweight ☐ 4. Obese ☐ 5. Not applicable ☐
11.	When you were between the ages of 30 to 35 years would you describe your weight as:	1. Underweight ☐ 2. Normal weight ☐ 3. Overweight ☐ 4. Obese ☐ 5. Not applicable ☐
12.	Would you consider your mother for most of your adolescent years to be:	1. Underweight ☐ 2. Normal weight ☐ 3. Overweight ☐ 4. Obese ☐ 5. Did not know her ☐
13.	Would you consider your father for most of your adolescent years to be:	1. Underweight ☐ 2. Normal weight ☐ 3. Overweight ☐ 4. Obese ☐ 5. Did not know him ☐
14.	How would you describe the weight of your living biological parents at present	
	14.1 Your father presently is	1. Underweight ☐ 2. Normal weight ☐ 3. Overweight ☐ 4. Obese ☐ 5. Did not know him ☐
	14.2 Your mother presently is	1. Underweight ☐ 2. Normal weight ☐ 3. Overweight ☐ 4. Obese ☐ 5. Did not know him ☐

Food Frequency Questionnaire

Instructions

- ❑ Look at the food item list (column 1)
- ❑ Think back carefully over the past month and determine how often you ate each item
- ❑ If you eat/drink a specific item less than once a month, mark the Never/<1/ month column.
- ❑ If you do eat/drink it more regularly, decide how often you eat it per month, OR per week, OR per day and make a cross (X) in the column which best applies to each item in the food list.
- ❑ Only make one cross (X) for each item in the list e.g. for each row in the table.

	Never/ <1/ month	1-3/ mth	1/ week	2-4/ week	5-6/ week	1/ day	2-3/ day	4-5/ day	6+/ day
STARCHES									
White or brown bread and/or buns/ rolls									
Whole wheat, health, Low GI, seed etc. bread and/or rolls									
Breakfast cereals or porridge such as All bran, high bulk bran, Muesli, Weet-bix, Pronutro, Oats etc.									
Breakfast cereals such as Rice crispies, Cornflakes, Coco pops, Fruit loops, Maize porridge etc.									
Rice, mealie rice, samp									
Pasta: macaroni, spaghetti, noodles									
Potato: cooked, baked, mashed									
Potato: cooked, baked, mashed with fat added or potato salad									
Legumes: baked beans, lentils, harricot beans, split peas, broad beans, kidney beans, sugar beans, dried bean salad/soup etc.									
VEGETABLES									
Cooked vegetables: any type. (no sugar/ fat/ sauce added)									
Vegetables: any type prepared with sugar/ fat/ sauces e.g. white sauce.									
Mixed salad: lettuce, cucumber, tomato, peppers, onions, mushrooms, carrots in any combination or alone.									
FRUIT									
Fresh fruit (any type)									
Dried fruit (any type)									

	Never/ <1/ month	1-3/ mth	1/ week	2-4/ week	5-6/ week	1/ day	2-3/ day	4-5/ day	6+/ day
Fruit juice									
Fruit salad: fresh or tinned									
MILK, YOGHURT AND CHEESE									
Full cream: milk, yogurt, sour milk (maas)									
Skimmed/ low fat/2%: milk, yogurt, sour milk (maas)									
Coffee creamer: in tea/coffee e.g. cremora									
Milk drinks: Milo, Nesquik, Horlicks									
Cheese: gouda, cheddar, camembert, brie, edam etc. (except low fat/ fat-free cottage cheese)									
MEAT, FISH, CHICKEN									
Snitzels, gorden bluer									
Red meat e.g. beef, mutton, pork. (Eat meat and visible fat)									
Red meat e.g. beef, mutton, pork. (Eat meat, but remove visible fat)									
Red meat e.g. venison & ostrich.									
Chicken/turkey: with skin									
Chicken/turkey: without skin									
Fried fish in any fat or oil, with or without batter/crumbs.									
Fish: steamed, grilled, braaied (fire)									
Fish: tinned sardines, pilchards, salmon, tuna									
Sausages: Vienna's, russians, frankfurter									
Cold meat: polonie, salami, etc. & bacon									
Eggs: cooked or poached									
Eggs: scrambled, baked, omelettes									
FATS									
Soft margarine									
Butter/hard margarine									

	Never/ <1/ month	1-3/ mth	1/ week	2-4/ week	5-6/ week	1/ day	2-3/ day	4-5/ day	6+/ day
Salad dressing, mayonnaise: normal fat									
Salad dressing, mayonnaise: lite/ low fat									
FAST FOODS AND TAKE AWAYS									
Pizza									
Pies & Sausage rolls									
Potato chips (French fries)									
Kentucky Fried Chicken									
Hamburgers (= bun and meat or chicken patty) e.g. McDonalds, Steers, Wimpy, Spur, other restaurants etc.									
OTHER									
Vetkoek, samoosas, koeksister, doughnuts									
Muffin, scones, cake, tart									
Rusks: commercial or homemade e.g. bran, buttermilk, white, whole wheat etc.									
Cookies: commercial or homemade: e.g. oat, crunchies, shortbread									
Chips: niknaks, simba etc.									
Energy bars, health bars, breakfast bars									
Chocolate									
Ice cream									
Cheese sauce, white sauce, meat sauces									
Tomato sauce, chutney, mustard, sweet chilli sauce									
Sweets e.g. jelly tots, sour worms, super-C's etc.									
Nuts and peanuts									
Peanut butter									
Chocolate spread									
Jam, syrup, honey									

	Never/ <1/ month	1-3/ mth	1/ week	2-4/ week	5-6/ week	1/ day	2-3/ day	4-5/ day	6+/ day
DRINKS									
Wine: red or white									
Port, sherry, liqueur									
Beer, cider, coolers e.g. castle, black label, hunters dry, savanna, smirnoff etc.									
Bier, cider, cooler diet/ light e.g. Savanna light									
Spirits: e.g. brandy, whisky, rum, vodka, gin.									
Fizzy soft drinks: e.g. coke, fanta									
Fizzy diet soft drinks: e.g. coke lite etc.									
Energy drinks e.g. energade, powerade									
Milkshake									
EATING PLACE									
In general, how often do you eat out e.g. restaurant, take-aways, hotel, prepared food/ meals from Spar, Checkers etc.									
If you work during the day (away from your home), how often do you take food from your home with you to eat during the day.									
If you work during the day (away from home), how often do you buy food to eat during the day.									