The role of fructose restriction in addition to dietary modifications for weight loss and lifestyle improvement, on fertility outcome and other markers of metabolic syndrome (MS), in obese women with polycystic ovarian syndrome (PCOS)

by

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Thesis presented in partial fulfilment of the requirements for the degree of Master of Nutrition in the Faculty of Medicine and Health Sciences at Stellenbosch University.

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DECLARATION

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ABSTRACT

The role of fructose restriction in addition to dietary modifications for weight loss and lifestyle improvement, on fertility outcome and other markers of metabolic syndrome, in obese women with polycystic ovarian syndrome (PCOS)

Introduction: At the time at which the current study was undertaken no data, as yet, existed on whether restriction of fructose, while treating obese patients with PCOS for weight loss, improves the clinical symptoms and metabolic/anthropometric profile so as to promote fertility.

Objectives: To evaluate the baseline intake of fructose, as well as the effect of restricting fructose intake from fruit and soft beverages to less than 20 g daily, as well as to provide guidelines for weight loss on anthropometric measurements, for improving subjective clinical symptoms, and for promoting fertility outcome in obese patients with PCOS, who seek to become fertile.

Methods: The study was conducted in the Tygerberg Hospital Infertility Clinic, as an experimental cohort. Patients with a body mass index (BMI) higher than 27, seeking fertility after diagnosis with PCOS, were referred for dietary consultation, and followed up 3 monthly over 1 year. At each visit anthropometric measurements and a detailed dietary history were taken and a questionnaire for clinical symptoms was completed.

Results: Baselinely, 86 patients were included in the study. Averages for weight and BMI were 99.8 ± 24.3 kg and 39.2 ± 8.7kg/m^2, respectively. Average baseline daily fructose intake was 167 ± 116.8g. At baseline, significant relationships were shown between fructose intake and burning feet (ρ=0.02) and frequent waking (ρ=0.02), with a trend towards nightly eating (ρ=0.07). The dropout rate after visit 1 was 50%, with a further dropout of 41% after visit 2.

After 3 visits (n=18), fructose intake significantly reduced (ρ=0.018), with the significant relationships with clinical symptoms having disappeared by visit 2. After 3 visits (n=18), both weight and BMI decreased significantly (ρ=0.017) and (ρ=0.019), respectively. Fructose was tested as a covariate to BMI, with high significance (ρ=0.006) in said population group.

Conclusion: Dietary intervention to reduce fructose intake proved significant for weight loss and BMI after 3 visits. Reduced fructose intake was associated with reduced clinical symptoms. With fructose being a significant covariate to BMI, it can be concluded that fructose overconsumption could possibly contribute to both clinical symptoms and elevated BMI in said study population.

OPSOMMING

Die rol wat die beperking van fruktose speel bykomend tot dieetaanpassings en lewenstylverbetering vir gewigsverlies by oorgewig vroue met polisistiese ovariële sindroom (PCOS) in die uitkoms van fertiliteit en ander merkers van metaboliese sindroom.

Inleiding: Met die aanvang van hierdie studie was daar is geen data beskikbaar oor die invloed van die beperking van fruktose in die dieet van oorgewig pasiënte met PCOS wat vir gewigsverlies behandel word nie. Dit was ook nie bekend of laasgenoemde pasiënte se kliniese simptome en metaboliese/antropometriese profiel sou verbeter met die beperking van fruktose sodat fertiliteit by hierdie pasiënte terselfdertyd ook bevorder word nie.

Doelwitte: Die evaluering van die aanvanklike inname van fruktose, sowel as die beperking van fruktose afkomstig van eetbare vrugte en versoete drankies en sap tot ’n inname van minder as 20 g daagliks, tesame met riglyne vir gewigsverlies. Die uitkoms hiervan is bepaal deur antropometriese metings, die verbetering in subjektiewe kliniese simptome en die fertiliteituitkoms by oorgewig pasiënte wat hulp met fertiliteit verlang.

Metodes: Die studie het as ’n eksperimentele kohort by die Infertiliteitskliniek by Tygerberg Hospitaal plaasgevind. Pasiënte wat na diagnose met PCOS fertiliteitsbehandeling verlang het en ’n BMI hoër as 27 gehad het , is vir dieetbehandeling verwys en driemaandeliks oor ’n tydperk van een jaar opgevolg. Tydens elke besoek is antropometriese metings en ’n omvattende dieetgeskiedenis geneem en ’n vraelys oor kliniese simptome ingevul.

Resultate: Aanvanklik is 86 pasiënte by die studie ingesluit. Gemiddeldes vir gewig en BMI was 99.8 ± 24.3 kg en 39.2 ± 8.7 kg/m^2 respektiewelik. Gemiddelde aanvanklike daaglikse inname van fruktose was 167 ± 116.8 g. Oorspronklik het betekenisvolle verhoudings tussen

fruktose en die volgende bestaan: brandvoete (ρ=0.02) en veelvuldige episodes van nagtelike wakkerheid (ρ=0.02), met ’n neiging na nagtelike etery (ρ=0.07). Die uitvalsyfer na een besoek was 50% met ’n verdere uitvalsyfer van 41% na die tweede besoek. Na drie besoeke (n=18) het sowel die gewig as die BMI betekenisvolle afname getoon (ρ= 0.017) en (ρ=0.019), respektiewelik. Fruktose is as ’n belangrike kovariant vir BMI (ρ= 0.006) vir hierdie populasiegroep geïdentifiseer.

Gevolgtrekking: Dieetintervensie vir die vermindering van die inname van fruktose was beduidend vir gewigsverlies en afname in BMI na drie besoeke. Verminderde fruktose-inname het gelei tot die vermindering van kliniese simptome. Met fruktose as beduidende kovariant vir BMI kan die gevolgtrekking gemaak word dat die oor-inname van fruktose by hierdie studiepopulasie waarskynlik tot sowel kliniese simptome as BMI bygedra het.

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LIST OF ABBREVIATIONS

Acetyl-CoA	acetyl coenzyme A
AGE	advanced glycation end product
ApoB	apolipoprotein B100
ASRM	American Society for Reproductive Medicine
BMI	body mass index
CCK	cholecystokinin
CNS	central nervous system
CT	computed tomography
CVD	cardiovascular disease
DM	diabetes mellitus
DNL	*de novo* lipogenesis
EGIR	European Group for the Study of Insulin Resistance
ESHRE	European Society for Human Reproduction and Embryology
GI	glycaemic index
GL	glycaemic load
GLUT-4	glucose transporter type 4
GLUT-5	glucose transporter type 5
HDL	high-density lipoprotein
HFCS	high fructose corn syrup
HFCS-42	high fructose corn syrup with 42% fructose
HFCS-55	high fructose corn syrup with 55% fructose
HFCS-90	high fructose corn syrup with 90% fructose
IDF	International Diabetes Federation

IGT	impaired glucose tolerance
IL-6	interleukin-6
IR	insulin resistance
LDL	low-density lipoprotein
MS	metabolic syndrome
NCEP	National Cholesterol Education Program
OGGT	oral glucose tolerance test
PCOS	polycystic ovarian syndrome
PFK	phosphofructokinase
PI-3-kinase	phosphatidylinositol 3-kinase
QOL	quality of life
SHBG	sex-hormone-binding globulin
SHREB-1c	sterol receptor binding protein-1-c
TNF-α	tumour necrosis factor-alpha
VLDL	very-low-density lipoprotein
WC	waist circumference
WHO	World Health Organisation
WHR	waist–hip ratio

CHAPTER 1

INTRODUCTION

Weight loss has been strongly suggested as a first-line therapy for overweight women with PCOS, seeking fertility.[1,2] Studies and research into the most effective dietary regime to bring about the desired weight loss have been largely ineffective,[3,4,5] and a higher dropout rate from dietary regimes has been seen in the overweight PCOS population, than in the weight-matched, non-PCOS controls.[6,7] The optimal dietary regime for PCOS still eludes scientist and researchers internationally.

The clinical, anthropometric and symptomatic profile of the overweight patient with PCOS is distinct, and both lean and overweight women suffering with PCOS can suffer debilitating symptoms, which affect their self-esteem and quality of life (QOL). PCOS has been referred to as "the thief of womanhood"[8].

The escalation of obesity around the world has led to the investigation of many causes of the problem, with excessive energy from fat intake and growing portion sizes receiving attention. Excess calorie intake is now being strongly linked with the intake of sweetened beverages, containing high amounts of sucrose and high-fructose corn syrup (HFCS).[9] Since sucrose contains 50% fructose, and the HFCS that is used in beverages contains 55% fructose, fructose intake could be suspected as being a major contributor in the obesity epidemic.[9]

The literature is clear on the fact that insulin resistance (IR) is a chief component for the development of PCOS in both lean and overweight women, and also forms the pathogenic link between PCOS and the MS (a cluster of obesity-related risk factors).[4,10,11]

Fructose baselinely drew interest as being a potentially useful sweetener for patients suffering with diabetes mellitus (DM), due to its lower glycaemic index (GI) as compared to glucose.[11]

The fact that fructose does not require insulin for the baseline steps of its hepatic metabolism was seen as an additional advantage.[11] Fructose consumption in humans has now been linked to the presentation of each of the features of the MS, including dyslipidaemia, visceral adiposity, IR and hypertension.[11]

There are key differences in the metabolic pathways that glucose and fructose follow. It is believed that the ability of the liver to metabolise high doses of fructose is responsible for the disruption in energy stores and fuel metabolism observed in excessive fructose intake. Of key importance is the ability of fructose to bypass the main regulatory step in glycolysis, namely the conversion of glucose-6-phosphate to fructose 1, 6-biphosphate, controlled by phosphofructokinase (PFK). Thus, while glucose metabolism is negatively regulated by PFK, fructose can continuously enter the glycolytic pathway, uncontrollably producing glucose, glycogen, lactate, and pyruvate, providing both the glycerol and acetyl portions of triglyceride molecules.[12] Such bypass can also result in increased glycogen deposition, *de novo* lipogenesis (DNL), and high production of lactic acid.[13,14]

1.1 RESEARCH PROBLEM

No data exist to date on whether restriction of fructose in particular, while treating obese patients with PCOS for weight loss, improves the clinical symptoms and metabolic/anthropometric profile of such patients. Previous research classified the type of carbohydrate simply through the GI,[3,5] making no distinction with regards to obese patients with PCOS between the key differences in the metabolic pathways of glucose and fructose. Through the extensive literature review in the current thesis, it is suggested that chronic fructose consumption leads to metabolic dyslipidaemia and other features of metabolic and IR syndromes. Dysregulation in hormonal appetite control has come to be shown to exist in patients with PCOS.[4,5,6,15,16] As is described in the literature study (Chapter 2), there is also

evidence to suggest that overconsumption of fructose leads to the same disturbances in appetite control hormones, increasing appetite, reducing satiety, and compromising meal termination in such patients.

To date, a PUBMED search has revealed no studies linking the detriments of high intake of fructose through drinks, juices and other fructose-sweetened foods to the aetiology or outcome of the metabolic profile or fertility of obese women with PCOS. Several means of search have been implemented, with none of them yielding any studies with fructose intake and the incidence/treatment of obese PCOS.

In the following chapter (Chapter 2), a literature review will be presented, in order to show the relevance between the condition of PCOS, with focus on the overweight/obese patient, to that of the fructose-overloaded patient, with regards to anthropometric and clinically measurable symptoms, as well as to the similarities in the metabolic profile of both conditions. The occurrence of IR in both profiles will be discussed, together with the possible mechanisms that bring about IR in both obese PCOS and in the fructose-overloaded patient. The similarities in both said causative factors for IR will be weighed up against each other to try and find common ground for both overweight/obese PCOS and fructose overload.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION TO POLYCYSTIC OVARIAN SYNDROME (PCOS)

PCOS presents as a heterogeneous condition, being the most common endocrinopathy that affects 5–10% of women during their reproductive years. The condition is characterised by chronic anovulation and hyperandrogenism, and, although it was previously thought of as only a fertility problem, it is now accepted as a metabolic disorder with serious health risks. Long-term consequences include increased risk for coronary heart disease, type 2 diabetes, hypertension, breast and endometrial cancer, as well as spontaneous abortion.[3,4]

Oligo-menorrhea or amenorrhea, anovulation, infertility, accelerated loss of scalp hair, hirsutism and acne form part of the classic features of said syndrome. Diagnosis of PCOS requires the presence of two out of three of the following symptoms, according to consensus reached by the 2003 Rotterdam European Society for Human Reproduction and Embryology (ESHRE) / American Society for Reproductive Medicine (ASRM)-sponsored PCOS consensus workshop group:

- oligo-/anovulation;
- biochemical and/or clinical signs of hyperandrogenism; and
- polycystic ovaries, with exclusion of other aetiologies, such as congenital adrenal hyperplasia, androgen-secreting tumours and Cushing's syndrome.1,4

With IR and compensatory hyperinsulinemia now recognised as a key factor in the pathogenesis of lean and overweight PCOS[4], reducing circulating levels and improving insulin sensitivity have become an essential part of management, in order to reduce the risk for developing type 2 diabetes and cardiovascular disease (CVD).[4,5,15,17] The definition of IR

is the inability of insulin to exert its biological effect, which manifests peripherally (at tissue level) or centrally (at hepatic level) through a reduction in the ability of insulin to lower plasma glucose.[15] The above is observed as impaired insulin-mediated uptake of glucose and suppression of lipolysis at the muscles or adipose tissue, the hepatic overproduction of glucose and the suppression of glycogen synthesis.[15]

2.1.1 Biochemical and metabolic profile of the overweight PCOS patient

As was previously mentioned, PCOS has been described as "the thief of womanhood"[15], and, together with fertility problems, hirsutism, oligo-/amenorrhea, IR and weight gain, the syndrome poses a serious hurdle to life quality in most sufferers. Hyperinsulinaemia causes increased androgen production by the theca cells of the ovaries, and reduces the action of hepatic sex-hormone-binding globulin (SHBG), with resultant hyperandrogenism (mainly free testosterone) and the features associated with PCOS.[1,3,8,15] Hyperandrogenism also correlates positively with IR, both in lean and overweight PCOS sufferers.[15] Although obese PCOS are considered to be more symptomatic and of greater health risk, PCOS sufferers with normal weight can also be at increased risk and suffer debilitating symptoms.[3] The current review focuses only on the obese PCOS patient seeking fertility, but all patients with PCOS should be investigated, and not only the overweight/obese.

In PCOS, follicular maturation is inhibited in the ovary, and hyperandrogenism occurs, resulting from increased ovarian androgen production and hypersecretion of the luteinising hormone (LH). Unlike skeletal muscle and adipose tissue, the liver and ovarian tissues do not appear to become insulin-resistant, and hyperinsulinemia synergistically stimulates the action of LH, causing androgen overproduction in the theca cells of the ovary.[15] The above leads to reduced hepatic production of sex-hormone-binding-globulin (SHBG), giving rise to elevated concentrations of both total and free androgens.[15] SHBG levels do not correlate with

androgen levels, but rather with IR and the body mass index (BMI).[8] SHBG levels are also predictive of the development of type 2 diabetes in women, and are shown to be similarly predictive of overall mortality in all post-menopausal women.[8]

An estimated 50–70% of women who suffer from PCOS are insulin-resistant, putting them at risk of developing type 2 diabetes and CVD.[4] The presence of PCOS seems to predispose women to the onset of impaired glucose tolerance (IGT) and type 2 diabetes, in both normal-weight and obese subjects, and there is an increased rate from normoglycemia to IGT and type 2 diabetes. Women with PCOS are also predisposed for increased risks of both gestational diabetes and miscarriage.[4] The risk of endometrial carcinoma in patients with PCOS is increased, especially in those who are insulin-resistant.[5]

Several studies have shown a higher incidence of cardiovascular risk in patients with PCOS.[4] The two predominant risk factors are elevated levels of triglycerides and low levels of high-density lipoprotein (HDL). Together with smaller low-density lipoprotein (LDL) particle size, hypertension and hyperinsulinemia, PCOS can be a strong predictor of CVD.[4] Obesity in PCOS is also associated with endothelial dysfunction and other markers of systemic inflammation, promoting development of CVD.[4,5] Elevated levels of both C-reactive protein and homocysteine have been shown in women with PCOS,[4] and in obesity, tumour necrosis factor-alpha (TNF-α) released from adipose tissue, as well as interleukin-6 (IL-6), which both have pro-inflammatory effects, in addition to increasing the risk of CVD.[5]

2.1.2 The association between PCOS and the metabolic syndrome (MS)

MS is defined as a "cluster of metabolic disorders that act synergistically to increase the risk of atherosclerosis"[10]. Obesity, and in particular abdominal/visceral adiposity and IR, act synergistically with the development of reproductive and endocrine abnormalities that

characterise PCOS.[18] IR is known to be a major contributor to / metabolic susceptibility factor in the development of MS.[5,10,18] Although the clinical relevance of MS has been questioned, evidence shows that its presence increases the risk of atherosclerotic vascular disease twofold, and the onset of DM fivefold.[10] PCOS has similarly been associated with an increased risk for both CVD and diabetes, and most of the symptoms of MS are also prevalent in PCOS. The suggestion has been made that PCOS might be a sex-specific form of MS.[10]

Evidence exists that women with MS tend to present with significantly higher free androgen concentrations and lower insulin sensitivity, which are both features of PCOS, compared with a control group of women without MS.[10] Although ovulation and menstrual regularity were not assessed, the findings support the notion of increased prevalence of PCOS amongst women with MS.[10] In PCOS, androgen excess seems to facilitate MS.[18] In female to male transsexuals, the administration of testosterone has been reported to promote visceral fat deposition, as has been the case in postmenopausal females.[18] A vicious cycle seems to exist between androgen excess and the deposition of visceral fat.[18] Although obesity and IR / compensatory hyperinsulinaemia are known as the two major denominators in the development of MS, neither is necessary or sufficient for the development of PCOS.[18] The two factors concerned appear to affect not only obese, but also lean, PCOS women, predisposing them to develop MS independently of obesity.[18] Women presenting with significant IR could have regular menses and normal levels of androgens.[18] The above might indicate that PCOS women tend to harbour an intrinsic theca cell defect, leading to ovarian hyperproduction of androgens, independently of extraovarian factors.[18]

Hyperinsulinaemia places an additional burden on the inherent ovarian dysfunction characterising PCOS.[18] In susceptible individuals, the degree of hyperandrogenaemia

increases with exacerbated IR / hyperinsulinaemia and the parallel courses between the two phenomena concerned possibly describes the association between testosterone and IR / hyperinsulinaemia.[18] However, even obese PCOS women might not seem to be insulin-resistant in terms of current methods, and thus PCOS and MS often coexist, but do not necessarily overlap.[18] The two syndromes represent two distinct clinical entities, which share common pathogenic mechanisms.[18] Androgen excess during intrauterine life results in a phenotype of the metabolic features of PCOS in animal models.[18]

At this point in the current thesis, it would be useful to describe the criteria that are used to define MS. The National Cholesterol Education Program (NCEP) (Adult Treatment Panel III) (2001), which provides the most commonly used definition for clinical purposes, states that at least three of the following must be present for a diagnosis of MS to be made:

- waist circumference (WC) > 100 cm in men; > 88 cm in women; or BMI ≥ 25 kg/m^2;
- triglycerides ≥ 1.7 mmol/l;
- HDL cholesterol ≤ 1.0 mmol/l in men; ≤ 1.3 mmol/l in women;
- Blood pressure ≥ 135 / 85 mm Hg, or on antihypertensive medication; and
- fasting plasma glucose ≥ 6.1 mmol/l.10,18

Two other definitions for defining or diagnosing MS come from the World Health Organisation (WHO) and from the European Group for the Study of Insulin Resistance (EGIR). Both the definitions concerned include IR or IGT, rather than the more simple impaired fasting glucose, as part of their criteria. Because of the laborious, but more accurate, nature of measuring IR, such as the hyperinsulinemic euglycemic clamp test and the intravenous glucose tolerance test, the WHO and EGIR definitions are better suited for research purposes than clinical practice.[10]

In 2005, the International Diabetes Federation (IDF) published a worldwide consensus definition of MS that can easily be applied in clinical practice, making provision for ethnic specificity of waistline circumference. One of the differences between the IDF and the NCEP definitions is that the IDF states that, if the BMI exceeds 30 kg / m^2, central obesity can be assumed, and the WC does not have to be measured. Also, the IDF uses geographically-specific cut-off points for WC, while the NCEP uses only one set of cut-off points for WC, regardless of geography. The IDF consensus worldwide definition of MS is stated as central obesity (defined as WC with ethnicity-specific values) or BMI ≥ 30 kg / m^2, in the presence of any two of the following:

- triglycerides ≥ 1.7 mmol/l, or specific treatment for this lipid disorder;
- HDL cholesterol ≤ 1.03 mmol/l in men, ≤ 1.29 mmol/l in women, or specific treatment for the lipid abnormality;
- systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 85 mm Hg, or treatment of previously diagnosed hypertension;
- fasting plasma glucose > 5.6 mmol/l. If above 5.6 mmol/l, the glucose tolerance test is strongly recommended, although it is not necessary to define the presence of MS.10,18

2.1.3 Body fat distribution in PCOS

Weight gain exacerbates IR, especially fat that is deposited in the abdominal region or android pattern / central obesity, where the waist–hip ratio (WHR) exceeds 0.85 in Caucasian/westernised women.[5,15] Such a phenomenon is commonly seen in patients with PCOS. Obesity, accompanied by abdominal obesity, worsens the features of menstrual irregularity and infertility, and is associated with anovulation and increased rate of spontaneous abortion.[5,15] Strong correlation exists between abdominal obesity and

hyperandrogenism, elevated levels of LH and IR.[15] Evidence exists, however, that abdominal visceral fat correlates more strongly with IR and with the features of MS, than with subcutaneous fat.[8,10]

Generally, 50% of females suffering with PCOS are overweight or obese, and have a higher incidence of diabetes and dyslipidaemia than do their non-PCOS counterparts.[5] The conclusion can, thus, be drawn that obesity, and especially android obesity, forms a complex interrelationship in the patient with PCOS, together with hyperandrogenism and IR. The syndrome has metabolic consequences that increase the risk of CVD through increased endothelial dysfunction, and that potentially increase the likelihood of the onset of type 2 diabetes sevenfold.[5] The accumulation of abdominal fat in PCOS is well documented, and can be measured by the WHR. The measurement concerned is, however, a crude one of visceral fat, and where more advanced imaging techniques were used, non-obese women with PCOS showed a higher percentage of total body fat in their upper body, and no difference in lower body fat compared with the controls.[8] Visceral fat mass is regarded as an aetiological feature in PCOS, and the features of IR and hyperandrogenism may be attenuated by dietary interventions aimed at reducing visceral fat. Such drug treatments as ovulation-induction agents act in a non-insulin-associated way, and do not alleviate metabolic abnormalities. Even with modest weight loss, the reduction in visceral fat mass is significant, which can lead to an improvement in metabolic parameters. WC, *per se*, is a more accurate way of assessing reduction in visceral fat, as WHR and BMI have been shown to have poor correlation to visceral fat rating.[10,19] Penaforte et al. used bioelectrical impedance to measure the weight, fat mass, and subcutaneous arm fat, together with trunk, neck and hip circumferences. Computed tomography (CT) was used to assess total abdominal fat, visceral fat and trunk fat. The study showed a good correlation between measured trunk circumference and assessment of trunk fat by CT. The researchers concluded that trunk circumference holds close

association with metabolic variables in PCOS and is a valuable tool for assessment of body fat distribution in obese women with PCOS.[20]

The mechanism by which visceral adipose tissue leads to hyperinsulinemia is probably through increased release of free fatty acids by visceral fat due to altered lipolysis, which drains to the liver via the portal vein and causes IR and hyperinsulinemia. In addition to the above, insulin exerts an anabolic effect on body fat distribution, and is very likely to play a role in the development of visceral obesity in PCOS.[10]

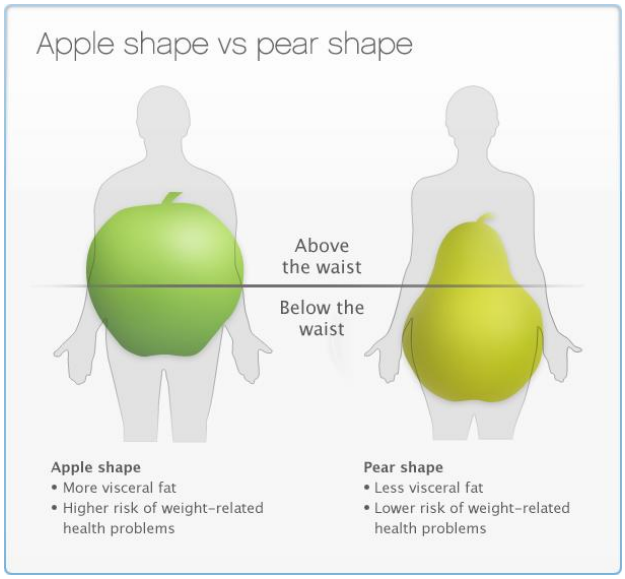

Figure 2.1: Body fat distribution (no © found)

2.1.4 Dietary treatment for PCOS

The basic goal for the treatment of PCOS is to restore fertility through normalising of serum androgen levels, which this can only be achieved when IR is reduced by decreasing the total body weight and the abdominal/visceral fat, measured as the waistline circumference of the patient.[15] Although several studies have alluded to weight loss as the cornerstone of dietary treatment for the obese PCOS patient, a myriad of different approaches have been suggested to achieve the desired weight loss, among which are the following:

- energy restriction;[3,4,5,8,10,15,17,20]
- modifications in macronutrient contents (differing ratios between fat, carbohydrate and protein);[8,17]
- reduction of the GI / load of carbohydrate intake;[3,4,15]
- decrease in the saturated and trans-fat contents, and modification of the monounsaturated fat content of the diet;[5,17,21] and
- meal replacement use as a short-term strategy.[22]

Weight loss prior to pregnancy also improves live birth rate in obese women, regardless of PCOS. Weight loss is internationally recommended and accepted as the first-line therapy in obese women with PCOS who wish to become pregnant.[2] The benefits of weight loss have been demonstrated in such conditions as diabetes and CVD, and obesity is well recognised for its association with poor fertility outcome. Several studies have shown that improved spontaneous ovulation is associated with as little as 5% weight loss in females who suffer from PCOS. Weight loss of 5–10% of baseline body weight has already been shown in studies to be associated with improved insulin sensitivity and reduction in circulating insulin levels, as well as reduction in hyperandrogenism with improvement of menstrual function, ovulation and fertility, in addition to hirsutism.[5,15] Although the reproductive function resumes with weight loss of as little as 10% of baseline body weight, the BMI of the PCOS sufferer having lost weight might still be above 30.[15]

Although recommended internationally as the first-line therapy in treatment of obese PCOS, the exact nature of the dietary manipulation in order to bring about the desired weight loss is still unclear.[2,4,15,17] A combination of therapies, including behavioural counselling, diet and exercise, pharmacological treatment and bariatric surgery, is suggested by the Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, which was held in Greece in

2007.[2] Multiple observations have been made of the fact that weight loss improves spontaneous ovulation and live birth rate, and pregnancies are reported with as little as 5% loss of baseline body weight. Energy restriction, increased physical activity, and agents to induce weight loss may be harmful to the foetus in the periconceptional period. Such interventions should probably not take place together with fertility treatment, but prior thereto, until the risk of the therapies for pregnancy are better understood.[2]

Chavarro et al.[21] conducted a trial lasting 8 years, including 18 500 subjects, and examined the association between the intakes of different types of fat and ovulatory infertility. The researchers found that the consumption of trans-fatty acids instead of carbohydrates, monounsaturates or omega-6 polyunsaturates was associated with greater risk of ovulatory infertility. Intake of trans-fatty acids is also associated with increased concentrations of inflammatory markers, and with greater IR and risk for type 2 diabetes. Trans fats are mainly found in commercially baked and fried foods. High intake of trans-fatty acids was also found to indicate less health consciousness, which is of concern in couples who are seeking fertility. Women planning to become pregnant should receive advice regarding their fat intake to improve their overall risk for CVD and diabetes, although responding well to such advice could improve their fertility as well.[21]

Improvement in insulin sensitivity and reduction of hyperinsulinaemia are recognised as the core reasons for bringing about improvement in metabolic and reproductive outcomes, which suggests that dietary manipulation that is designed to improve IR might be of greater benefit than merely restricting energy intake.[4,5,17] Increasing evidence suggests that diets with a reduced glycaemic load (GL) might be beneficial in reducing hyperinsulinaemia and its associated consequences.[2,3,4,5] The glycaemic load of carbohydrates is defined as the amount of carbohydrate in a particular food, multiplied by the GI of that food.[5] GI can be seen as a

type of carbohydrate, while GL can be seen as the amount of dietary carbohydrate consumed. Altered dietary composition may influence insulin sensitivity, even without weight loss. A reduction of the GL may reduce postprandial glycaemia and the resultant hyperinsulinaemia. The best way to bring about a reduced GL remains unclear, although it could be either of the following two ways: reducing the GI of carbohydrate intake; or cutting down on the total amount of carbohydrate consumed.[4]

Jenkins et al.[23] developed and introduced the concept of GI to facilitate the classification of carbohydrates according to their postprandial glycaemic effect, although the classification was based on a very small subject sample (n=34)[23]. The main purpose with the concept of GI was to improve glycaemic control in diabetics, and a meta-analysis by Brand-Miller et al. confirmed the benefits to be obtained thereby.[4] Moran[6] reports that the modification of the type of dietary carbohydrate or GI has been highly controversial, and that high carbohydrate diets may worsen the metabolic profile if no weight loss is achieved. Although data exist that lowering the GI of dietary carbohydrate improves satiety, the evidence for weight loss is poor. In addition, there appears to be no evidence of the usefulness of GI as a strategy for weight management in women with PCOS.[6]

In contrast, some evidence suggests that a higher ratio of protein to carbohydrate for weight loss regimes holds some metabolic benefit. There is sound evidence that the higher satiety derived from a higher intake of protein assists in weight loss over time, and leads to a lower GL, due to reduction in carbohydrate intake.[19,24] Traditionally, diets that are high in carbohydrate and low in fat have been used to bring about weight loss and to improve metabolic and reproductive function, but there has been increased interest in the use of low carbohydrate / high protein diets.[25] In comparison to both fat and carbohydrate, protein may assist in weight loss, due to its superior satiating power, and may improve lean body mass

due to weight loss, with improved insulin sensitivity.[5,19,24,25] Postprandial thermogenesis is also increased by protein intake, which could have favourable effects on abdominal fat.[5] In 2005, a higher protein intake regime from lean red meat and dairy sources, restricted in energy, showed a weight loss advantage in patients suffering with elevated triglycerides, which is considered a strong marker for the presence of MS. The researchers found that subjects who consumed 30% protein from an energy-restricted diet reported experiencing less hunger than did those on a high-carbohydrate regimen. In addition, there were no detrimental effects on bone or renal metabolism of the higher protein regime over 12 weeks, and no difference between dietary composition (high protein as opposed to high carbohydrate, both with energy restriction) with effect to LDL cholesterol, HDL cholesterol, and glucose concentrations.[24] Studies elsewhere have also shown that low-fat, energy-restricted diets with higher protein content have a greater reducing effect on triglycerides, although weight loss was reported as being equal to that gained through the standard protein diet.[4]

Larsen and co-workers from the University of Copenhagen conducted a study on overweight adults from eight different European countries. The participants first had to lose 8% of their baseline body weight by means of going on a low calorie (800–1000 kcal) diet, and were then randomly assigned to one of five *ad libitum* diets for 26 weeks to prevent regain.[26] Apart from the control, the diets were low protein and low GI, low protein and high GI, high protein and low GI, and finally high protein and high GI. The high-protein and low-GI diets showed better outcome in terms of weight maintenance than did the low-protein and high-GI diets. The researchers reported that, after the baseline weight loss, the participants following the high-protein and low-GI diet continued to lose weight. The team also concluded that, since the higher protein content was achieved through reduction of the carbohydrate content, further support was granted the notion that, by lowering the GL of the diet (defined as

carbohydrate content multiplied by the GI), body weight in obese patients can be better controlled.[26]

2.1.5 Weight maintenance and dropout

The sustainability of weight reduction diets in obese women with PCOS is well known to be poor over the long term, with little maintenance of weight loss.[7] Moran et al.[6] substantiate the view by referring to the 26–38% dropout rate for PCOS sufferers on weight-loss regimes, as opposed to 8–9% in non-PCOS subjects. Although anecdotal reports of increased difficulty with weight loss in PCOS abound, the phenomenon has never been scientifically proven.[6] The high dropout rate among PCOS sufferers might be due to abnormal appetite regulation, leading to difficulty with energy restriction. This implies that PCOS is a population that requires intensive long-term dietary coaching, with regular follow-up and support. Dietary strategies to maximise satiety are certainly applicable to help the patients to achieve a desirable body weight.[6]

In a study by Douglas et al.[27] that was aimed at analysing the dietary composition of women with PCOS as compared with healthy, control-group women, the researchers concluded that PCOS sufferers showed a greater tendency towards eating more junk food with a higher GI than did the control group. In 2008, Humphreys and Costarelli[28] reported that 9 out of 35 overweight women with PCOS had actually been referred to a dietician. The researchers concluded that the support given to reduce and maintain weight in the group was inadequate, and required improving.

Huber-Buchholz et al.[7] allude to the fact that many studies have shown and recommended weight loss as an effective method for the induction of ovulation in obese women with

menstrual disturbances. However, the diets concerned are associated with a poor compliance rate over the long term, with little maintenance of weight loss.

2.1.6 Disturbances in hormonal regulation of appetite in PCOS

That derangements in appetite control hormones exist in patients with PCOS is supported by emerging evidence. Moran and co-workers[16] have previously shown that postprandial satiety is lower and that postprandial hunger is higher, both before and after weight loss, than in weight-matched controls. Evidence does exist that disturbances in appetite regulation in PCOS probably account for the reported disturbances in hunger and satiety signals.[4,5,6,15,16]

2.1.6.1 Cholecystokinin (CCK)

The hormone CCK is released in the duodenum in response to postprandial protein and fat, and delays gastric emptying, thereby increasing satiety and reducing meal size and caloric intake, and assists with meal termination. Compared with weight-matched controls, there is a reduced postprandial CCK response in overweight women with PCOS, which suggests dysregulation of appetite control by CCK in PCOS.[6,16]

2.1.6.2 Ghrelin

Ghrelin is an orexigenic hormone that is produced in the fundus of the stomach.[5,6,15,16] Levels of ghrelin increase sharply in anticipation of a meal, and again decrease 2–3 hours after the meal, stimulating hunger through action on the hypothalamic arcuate nucleus and producing satiety after meal consumption.[6,15,16,29] In obese subjects with or without PCOS, fasting levels of ghrelin are decreased, and postprandial suppression of ghrelin is impaired, causing diminished satiety after meals and compromised meal termination.[6,15,29] That the insulin and glucose responses to meals contribute to suppression of ghrelin after meals and to the ingestion of glucose, but not to that of water or protein, producing a reduction in circulating

ghrelin by 30–50% is supported in the literature.[29,30] Although the down-regulation of ghrelin in obesity might be improved by weight loss and fasting levels of ghrelin improved, the restoration of ghrelin homeostasis seems to be impaired in PCOS.[6,15,16,29]

In a study by Scöfl et al.,[30] 26 women with PCOS were compared to 61 healthy controls. Serum levels of ghrelin in the PCOS group were found to be significantly lower than in the control group (even matching the ghrelin levels found in gastrectomised women), and strongly relating to the degree of IR.[30] Apart from appetite control, ghrelin also has a variety of other functions, such as in exocrine pancreatic function, glucose metabolism and control, increased inflammation and vasodilatation, and ovarian function. The lower fasting ghrelin levels seen in PCOS subjects might also be a reflection of the increased metabolic, diabetic and reproductive dysfunction characteristic to said condition, rather than abnormality in appetite regulation.[16] The amount of weight loss that is required to bring about ghrelin restoration is also unknown.[6] An inverse relationship exists between circulating levels of ghrelin and body weight,[29] which suggests that increased ghrelin through weight loss might contribute to hunger and weight gain.[29] The impaired satiety through down regulation of fasting ghrelin and impaired postprandial ghrelin suppression might explain the difficulty in adhering to weight-loss regimes that is experienced amongst PCOS women, and long-term weight loss regimes should be based on strategies to improve satiety. Regular follow-up and support are considered crucial in the weight management of overweight or obese subjects with PCOS.[6] Increased postprandial ghrelin seen in patients after a regime of diet and exercise to bring about weight loss is not seen in patients who have lost weight as the result of bariatric surgery. The factor is most probably one that contributes to the high success achieved in use of said procedure to produce sustained weight loss.[29]

2.1.6.3 Insulin

By way of its actions in the central nervous system (CNS) to inhibit food intake and to increase energy expenditure, insulin serves as a potent regulator of body adiposity, and insulin receptors are widely expressed in several areas of the CNS involved in the control of food intake and energy expenditure. Insulin is secreted in response to glucose-containing carbohydrates and some amino acids, as well as to the incretin hormones GLP-1 and GIP. Insulin inhibits food intake by activation of phospatidylinositol 3-kinase (PI-3-kinase), a pathway that is shared with leptin, which also has anorexigenic effects. Evidence is clear from both human and animal studies that reduced delivery of insulin into the CNS, or disruption of CNS insulin signalling, results in weight gain and obesity.[29]

Due to its well-described peripheral anabolic effects to stimulate lipid synthesis and storage, the misconception that insulin causes weight gain and obesity has arisen, leading to the promotion of such fad diets as very low carbohydrate diets and low-glycaemic-index diets. The diets are based on the notion that weight loss can be achieved simply by avoiding foods that stimulate insulin secretion. The difference between insulin responses to meals, when circulating levels of insulin rapidly increase and return to baseline, and chronic hyperinsulinemia, which is a secondary effect of beta cell adaptation to IR, is important to note.[29] Although chronic hyperinsulinemia is known to increase hepatic lipogenesis and to contribute to hypertriglyceridemia, no direct connection between hyperinsulinemia and weight gain has yet been established. Possibly, the peripheral anabolic effects of insulin are unopposed in the presence of central insulin and leptin resistance, which leads to weight gain through lipogenesis. Regarding the GI of dietary carbohydrate, a study in 2002 reported that carbohydrates with a higher GI (larger glucose excursion), which would have been expected to have a larger insulin stimulation, actually resulted in lower short-term appetite ratings and in a decrease of *ad libitum* food intake after one hour. [29]

Despite the peripheral effects of hyperinsulinemia, little evidence exists to imply that insulin responses to meals alone cause overweight and obesity. Reduced insulin responses to meals have been shown to be predictive of future weight gain and of subsequent increases in visceral fat in Pima Indians and Japanese Americans, respectively. Thus, insulin responses to meals are in fact protective against, rather than contributory to, weight gain and obesity. Moreover, the insulin response to meals is an important mediator of leptin production by adipose tissue.[30]

2.1.6.4 Leptin

Leptin, an anorexigenic hormone produced by adipocytes, together with insulin serves as a critical endocrine signal to the CNS to regulate food intake, energy expenditure and body adiposity. As was earlier mentioned, the actions of insulin and leptin share a common signalling pathway via activation of PI-3-kinase. In humans, defects in the ability to produce leptin or the leptin receptor lead to severe hyperphagia and obesity. In such leptin-deficient patients, administration of leptin markedly reduces appetite and body fatness. The reduction of circulating concentrations of leptin during periods of dieting is related to the amount of hunger that is experienced by dieting women, which probably accounts for their lowered metabolic rate and weight regain.[29]

Leptin levels decline during periods of fasting or caloric restriction to a much greater degree than would be expected from the small amount of body fat lost. Since plasma levels of leptin only increase after 4 hours postprandially, leptin is not regarded as a short-term signal for satiety, but, rather, that leptin acts as a medium- to long-term regulator of energy balance. Leptin concentrations demonstrate a diurnal pattern, with a nadir (low level) midmorning, and a peak between midnight and 02h00. The diurnal pattern does not occur in fasting subjects, but is entrained by meal timing, and is directly related to insulin responses to meals.

The presence of insulin increases both leptin gene expression and leptin secretion, and is the key signal in the regulation of leptin production and its diurnal pattern. High-fat meals, resulting in smaller insulin and glucose excursions postprandially, have been noted to reduce significantly the production of leptin over a 24-hour period, and the amplitude of the diurnal leptin peak was blunted, compared to that which was experienced with low-fat, high-carbohydrate meals.[29]

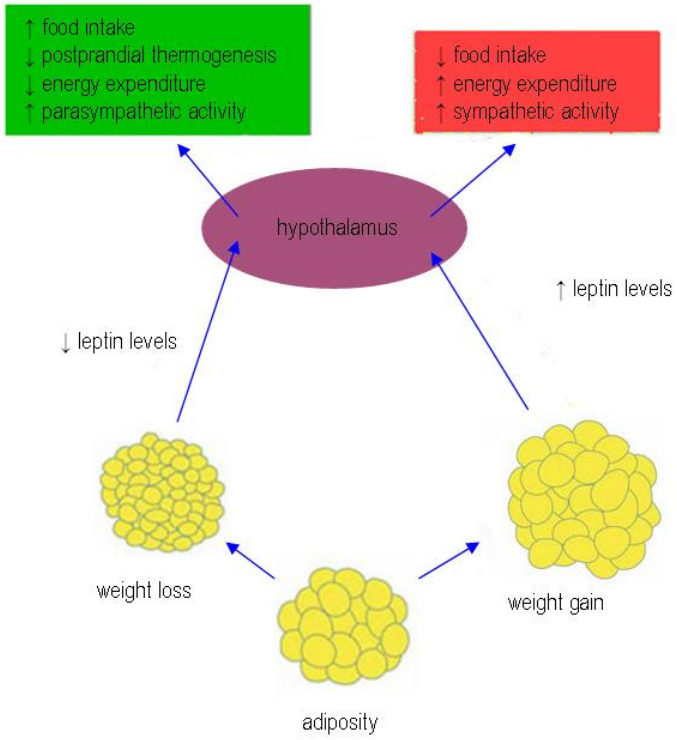

Figure 2.2: Example of the pathway of leptin (adapted from [12,31])

Evidence exists that breakfast omission is associated with decreased insulin sensitivity within a short period of time (2 weeks), so that the importance of breakfast may not only lie in better energy distribution and thermic response to food. The most effective meal frequency to bring about weight loss is unknown, but a regular eating pattern with minimal snacking seems most desirable.[5] Increased levels of ghrelin in anticipation of food is a learned response, and, bearing in mind that ghrelin is the most orexigenic hormone, there is a good argument for regular, but not too frequent, meals in subjects who wish to lose weight.[5]

2.1.7 Subjective clinical symptoms

A multitude of symptoms have been described in overweight patient suffering from PCOS, with debilitating effects on every aspect of their lives.[3] When treating PCOS, it is useful to understand the broader clinical symptom profile of each patient, and not just the overweight involved.[3] Studies have shown that subjects with PCOS have higher levels of stress, and tend to experience a more negative self-image than do the controls.[19] Interest has recently been shown in the health-related QOL for the patients, and questionnaires have been developed, mostly based on mood and emotional issues.[19] Since lifestyle and dietary modifications should address behaviour in the course of therapy, a better understanding of the psychological background of the patient is crucial, while managing diet and lifestyle.[19] Thecal androgen production is stimulated by hyperinsulinemia, and the amount of SHBG is decreased, resulting in the hyperandrogenic clinical features of PCOS. At least 10–50% of PCOS patients are overweight or obese, and since weight gain exacerbates IR, the accumulation of fat in the abdominal area is pertinent.[3]

Environmental factors play a vital role in the expression of PCOS with IR and in the subsequent hyperinsulinemia, which is now recognised in the pathogenesis of PCOS. In Herriot et al.'s retrospective study, an audit was conducted in a private setting where patients were managed by either an gynaecologist or an endocrinologist, and then referred to a specialist dietician for a 90-minute consultation. Patients were advised on general healthful eating, as well as to lower both the GI and the GL of their carbohydrate intake to no more than 40–45% of total energy, to increase their protein intake to 30% of total energy, to reduce their intake of saturated fat, and importantly, to avoid snacking.[3] Overweight and obese patients were advised on overcoming calorie excess by increasing the vegetable proportion of their diet and on participating in moderate physical activity of 30 minutes per day. No control was exerted over the medicines prescribed to the patients concerned.

In the above study, 70% of patients baselinely reported carbohydrate cravings, with the incidence of such cravings being similar amongst lean and overweight PCOS patients. Lean patients experienced hypoglycaemia more frequently than did overweight patients (73% as opposed to 43%, respectively). Central weight gain was reported in 93% of overweight subjects, as opposed to 47% in lean patients. Tiredness and lethargy was reported in 82% of all patients in the survey. Symptoms of irritable bowel were reported in 68% of both patients, with or without obesity/overweight.

After dietary intervention, 50 patients were available for follow-up and 52% reported a reduction in carbohydrate craving, with the figure being similar to that which was reported by the lean subjects. Normal-weight patients reported a reduction in hypoglycaemia from 73% to 11%. Tiredness and lethargy were reduced to 29% in lean subjects, and to 40% in the overweight subjects, respectively.[3]

In the study, 40% of patients baselinely had a very low level of physical activity (< 90 minutes of moderate activity per week). Follow-up data were only available on 59 patients of the 88 who baselinely were included. In terms of post-dietetic intervention, only seven patients had data available for physical exercise, of which only three showed improvement or an increase in the amount of physical activity undertaken.[3]

QOL plays a key role in the life of a PCOS sufferer, with the condition already having been labelled “the thief of womanhood”[15], as has already been noted earlier in the current thesis. The symptomatic presentation of the patient, together with frustration regarding the weight gain and the loss of previous feminine characteristics, forms an important whole in the treatment of PCOS. The inability to produce children further exacerbates the condition, with the patient coming to rely on empathy and support from the clinician, whatever the discipline might be.

The next section (section 2.2) of the current thesis presents an introduction to the metabolic effects of fructose consumption, and has striking similarities to the MS, IR and PCOS.

2.2 THE EFFECT OF CHRONIC FRUCTOSE CONSUMPTION ON THE DEVELOPMENT OF INSULIN RESISTANCE AND THE METABOLIC SYNDROME

IR is often linked to the macronutrient content of the diet, and, in the past, diets high in saturated fats have been shown to induce weight gain, IR and hyperlipidemia in both animals and humans. Several recent reviews agree in their conclusion that, while there is strong evidence that diets high in fructose can produce obesity, IR / glucose intolerance and dyslipidaemia in animals, direct experimental evidence that chronic consumption of fructose promotes the development of the MS in humans is equivocal.[12,13,29,31]

2.2.1 Differences in metabolic pathways of glucose and fructose

Key differences in the metabolic pathways that glucose and fructose follow are apparent (Figure 2.3). The ability of the liver to metabolise high doses of fructose is believed to be responsible for the disruption in energy stores and fuel metabolism that is observed in excessive fructose intake. Of key importance is the ability of fructose to bypass the main regulatory step in glycolysis, namely the conversion of glucose-6-phosphate to fructose 1.6-biphosphate, which is controlled by phosphofructokinase (PFK). Thus, while glucose metabolism is negatively regulated by PFK, fructose can continuously enter the glycolytic pathway, uncontrollably producing glucose, glycogen, lactate, and pyruvate, providing both the glycerol and acetyl portions of triglyceride molecules.[12,32] The bypass can also result in increased glycogen deposition and in DNL.[13,14,32]

Other differences are present in the metabolism of fructose and glucose.[9,29] The transport mechanism of glucose into cells is known as glucose transporter type 4 (GLUT-4), and is insulin-dependent in most tissues. Once insulin has activated the insulin receptor, the density of glucose transporters on the cell surface rapidly increases, and facilitates entry of glucose into the cell. Next, glucose is phosphorylated to glucose-6-phosphate by glucokinase, and the intracellular metabolism of glucose begins. Through modulation by phosphofructokinase, the conversion of glucose-6-phosphate to the glycerol backbone of triglycerides can be tightly controlled. As opposed to glucose, fructose is transported into hepatic cells via a non-insulin dependent mechanism, glucose transporter type 5 (GLUT-5). No GLUT-5 transporters are present in brain tissue and in the beta cells of the pancreas, indicating limited entry of fructose into the tissues concerned.[9,13,29] Previous studies have indicated that fructose, unlike glucose, at most has a weak ability to stimulate insulin from the beta cells of the pancreas.[31] If fructose is given as part of a mixed meal, the rise in serum-glucose and insulin levels is smaller than it is, should an equal amount of glucose have been given.[9]

The fructose-specific hexose transporter, GLUT-5, is primarily expressed in the jejunum on both the brush border and on the basolateral enterocyte membranes, as well as in the lower levels in the kidney, skeletal muscle and adipocytes. In the case of large fructose consumption, the capacity of GLUT-5 to absorb fructose is exceeded, and diarrhoea can result. Intake of glucose together with fructose, as it should usually be consumed in beverages and with meals, seems to enhance fructose absorption. Some adaptation to high fructose intake takes place, as fructose absorption is increased during sustained high fructose intake.[29]

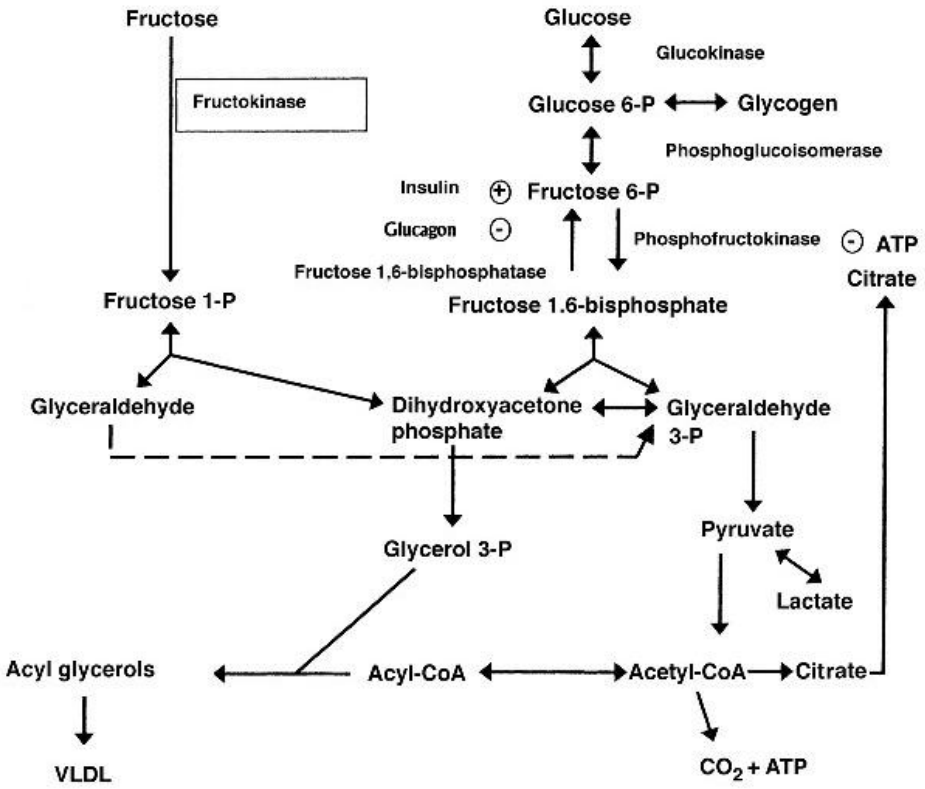

Figure 2.3: Different hepatic pathways of fructose and glucose[29]

2.2.2 Fructose contents of different sources

Sucrose (table sugar) consists of 50% glucose and 50% fructose, with the latter not being an essential sugar for the human body. The percentage of fructose in high-fructose corn syrup (HFCS)-sweetened beverages and sports drinks can be substantial, since HFCS contains between 42%, 55% and 90% fructose (HFCS-42, HFCS-55, and HFCS-90).[9,12,29] Although produced and widely used in many countries worldwide, the use of HFCS increased with 1000% in the United States between 1970 and 1990, with the country concerned being the largest user of HFCS worldwide.[9,12] Scant data exist on foods containing HFCS, other than in the United States.[9] The starch in corn can be effectively converted to glucose and from there to various amounts of fructose, using a glucose isomerase.[9] Corn-based syrups are inexpensive and have made it more profitable to replace sucrose and other simple sugars with HFCS, which now represents up to 40–43% of added caloric sweetener in the United States, being largely used in soft drinks and other sweetened beverages.[9,29]

The sweetness of fructose is 1.73 times more than that of sucrose.[9] If, for comparative purposes, the sweetness of sucrose is set at 100, the sweetness of glucose is 74.[9] Replacing sucrose with HFCS in soft drinks impacts on the ratio of fructose to glucose, as HFCS-55 has a fructose : glucose ratio of 1.22, with 10% more fructose, by weight, than sucrose.[29] Estimations are that 60% of the HFCS used in sweetened beverages comes from HFCS-55 and 40% from HFCS-42.[9,29] In the combined use of sucrose, HFCS-55 and HFCS-42, the average fructose content of sweetened beverages can reasonably be approximated at 50%.[29]

Crystalline fructose with a purity of 100% is also used to sweeten some foods and beverages, but scant data on the use of crystalline fructose are available.[29] Together with the use of sucrose as added sweetener, it could be estimated that the total intake of added fructose, plus naturally occurring fructose in fruit and fruit juices, is as much as 12% of total energy intake per day.[14,29] The estimate includes consumption of not only sweetened beverages, fruit and fruit juices, but also sweets and desserts. Based on a daily energy intake of 2000 calories per day, the fructose intake would be estimated as being at least 60 g/day.[29] Actual consumption of fructose is likely to be underestimated, due to selective underreporting of specific foods and drinks,[14,29] with certain population groups in the United States being likely to be consuming well over 100 g fructose daily from added sweeteners.[29]

Fruit juices differ widely in their content of fructose, with apple juice containing more than 60% of its caloric content in the form of fructose, and orange juice only 40–45%.[9,29] Apple- and other juice-sweetened juices and beverages contain higher amounts of fructose as a percentage of the total caloric content than do soft drinks sweetened with HFCS-42 or HFCS-55.[29] An increasing number of beverages and juice products are being sweetened with apple and white grape juice, resulting in a higher number of calories being provided by fructose.[9,29]

Investigators have linked the consumption of particularly apple juice, because of its popularity, to overweight and obesity in children aged 2–5 years.[33] In 1999, Dennison et al.[33] showed that the incidence of overweight in children who consumed more than 360 ml of any fruit juice per day was significantly higher than it was in those who consumed less. Dennison suggests that the increase in body weight seemed to be related to apple juice only, which has a high content of fructose.[33] Other consecutive studies refuted the findings, although a recent prospective cohort of Mediterranean adults showed a weak, but significant, association between weight gain and sweetened fruit juice consumption.[33] Since fruit juice remains an important source of nutrition of vitamins and minerals for children, further research of the association between fruit juice consumption and weight gain is warranted.[33]

Table 2.1: Carbohydrate composition (%) of commercial sweeteners[34]

Sugar	Fructose	Glucose	Other saccharides
HFCS-42[1]	42	53	5
HFCS-55[2]	55	41	0
HFCS-90[3]	90	5	0
Sucrose	50	50	0
Honey	49	43	8
Apple juice	59	31	10
Orange juice	51	49	0

1 - HFCS-42: high fructose corn syrup containing 42% fructose
2 - HFCS-55: high fructose corn syrup containing 55% fructose
3 - HFCS-90: high fructose corn syrup containing 90% fructose

2.2.3 Fructose: A highly lipogenic nutrient

When small amounts of glucose are infused into the portal vein, hepatic uptake of glucose improves, probably due to the stimulation of glucokinase. Glycogen synthesis is also stimulated by increased carbon flux, by means of glycogen synthase, and, apart from

stimulating glycogen synthesis, the above also restores the ability of hyperglycaemia to regulate the production of glucose in the liver.[29] Hence, small amounts of fructose seem to act in a catalytic way to improve hepatic glucose uptake and storage as glycogen, mainly because of the stimulation of hepatic glucokinase.[29] The fact was verified in an oral glucose tolerance test (OGGT) in which 10% of the glucose (75 g) was added as fructose (7.5 g). The outcome showed favourable results in adults with type 2 diabetes, suggesting that limited amounts of fructose would be useful in improving glycaemic control in type 2 diabetes.[13,29]

The liver is capable of metabolising fructose.[12] For millennia, the human body has been given fructose to the amount of 16–20 g per day from fresh fruit.[12] From Table 2.2, it should be clear that, apart from dried fruit such as figs, any three fresh fruits can be eaten on a daily basis, without exceeding the above-cited amount of 16–20 g. Westernisation of diets has resulted in significant increases in added fructose, leading to typical daily consumptions of 85–100 g per day.[12,13] In Vos et al.'s[14] determination of the fructose intake amongst US children, adolescents and adults, the highest intake (72.8 g / day) was found to take place amongst adolescents who were 12–18 years of age. The exposure of the liver to such large quantities of fructose leads to stimulation of lipogenesis and to rapid triglyceride accumulation, which, in turn, contributes to reduced insulin sensitivity and to hepatic IR / glucose intolerance.[12]

Table 2.2: Sugar content of selected common fruit and vegetables (g / 100 g)[35]

Food item	Total carbohydrate	Free fructose	Free glucose	Sucrose	Fructose/ glucose ratio
Fruits					
Apple	13.8	5.9	2.4	2.1	2.0
Apricot	11.1	0.9	2.4	5.9	0.7
Banana	22.8	4.9	5.0	2.4	1.0
Fig, dried	63.9	22.9	24.8	0.07	0.93
Grapes	18.1	8.1	7.2	0.2	1.1
Peach	9.5	1.5	2.0	4.8	0.9
Pear	15.5	6.2	2.8	0.8	2.1
Pineapple	13.1	2.1	1.7	6.0	1.1
Plum	11.4	3.1	5.1	1.6	0.66
Vegetables					
Beet, red	9.6	0.1	0.1	6.5	1.0
Carrot	9.6	0.6	0.6	3.6	1.0
Corn, sweet	19.0	1.9	3.4	0.9	0.61
Red pepper, sweet	6.0	2.3	1.9	0.0	1.2
Onion, sweet	7.6	2.0	2.3	0.7	0.9
Sweet potato	20.1	0.7	1.0	2.5	0.9
Sugar beet		0.5	1.0	16 - 17	1.0
Sugar cane		1.0	1.0	11 - 16	1.0

In an interim report on an on-going investigation comparing fructose and glucose intake of 25% of total energy through beverages, Stanhope and Havel found that, in particular, the high fructose group showed development of three of the pathological characteristics of MS: dyslipidaemia; IR; and increased visceral adipose tissue.[31] The researchers further report that, in older adults, as well as in shorter-term studies in younger adults, hypertriglyceridemia seems to be the earliest metabolic perturbation following high- fructose consumption.[31]

Current literature provides considerable evidence in support of the ability of high-fructose diets to up regulate the lipogenesis pathway, which, in turn, leads to increased triglyceride production.[12,31] The most likely cause of postprandial hypertriglyceridemia is increased hepatic DNL, which stimulates very-low-density lipoprotein (VLDL) production and exit from the liver. Hepatic lipogenesis is promoted by high-fructose consumption in three ways:

- Fructose is mainly metabolised in the liver.
- Fructose enters into the glycolysis pathway via fructose-1-phosphate and hence bypasses the main rate-controlling step of glycolysis catalysed by phosphofructokinase, providing unregulated amounts of acetyl coenzyme A (acetyl-CoA) and glycerol-3-phosphate, which are both lipogenic substrates.[12,13,31]
- Fructose is able to activate sterol receptor-binding protein-1c (SREBP-1c) independently of insulin, which, in turn, activates genes that are largely involved in DNL.[31]

Insulin and glucose directly regulate lipid synthesis and secretion.[12] SREBP-1c is a key transcription factor that is responsible for regulating fatty acid and cholesterol biosynthesis, and insulin controls its expression.[12] In all three major target insulin tissues of the body, namely liver, fat, and skeletal muscle, expression of SHREBP-1c is enhanced by insulin. Similarly, under conditions of IR with resultant hyperinsulinemia, SREBP-1c is enhanced. Under conditions of insulin depletion, namely through streptozotocin treatment, SREBP-1c is still expressed upon glucose, fructose or sucrose feeding. Together with the reduced insulin availability, it would have been reasonable to expect SREBP-1c down regulation, but such is not the case. With glucose feeding, a short-term peak of SREBP-1c is induced, with fructose being responsible for a gradual prolonged increase in SREBP-1c activity. The above proves

that, independent of insulin signalling, carbohydrate, particularly fructose, availability can bring about lipogenesis.[12,31]

The mechanisms for fructose-induced IR share similarities with those that promote high-fat-induced IR.[32] Neither fat nor fructose elicits insulin responses, and neither interferes with insulin signalling at common points in skeletal muscle. In hepatic cells, both high-fructose and high-fat diets elicit hepatic stress responses that activate inflammatory cascades.[32]

Intrahepatic VLDL production and secretion require the presence of available lipid substrate, which can be provided in unregulated amounts (acetyl-CoA and glycerol-3-phosphate) by entry of fructose into the liver.[31] The assembly of triglyceride into VLDL is dependent upon apolipoprotein B100 (ApoB), which is considered highly atherosclerotic (see Figure 2.4).[12,29,31]

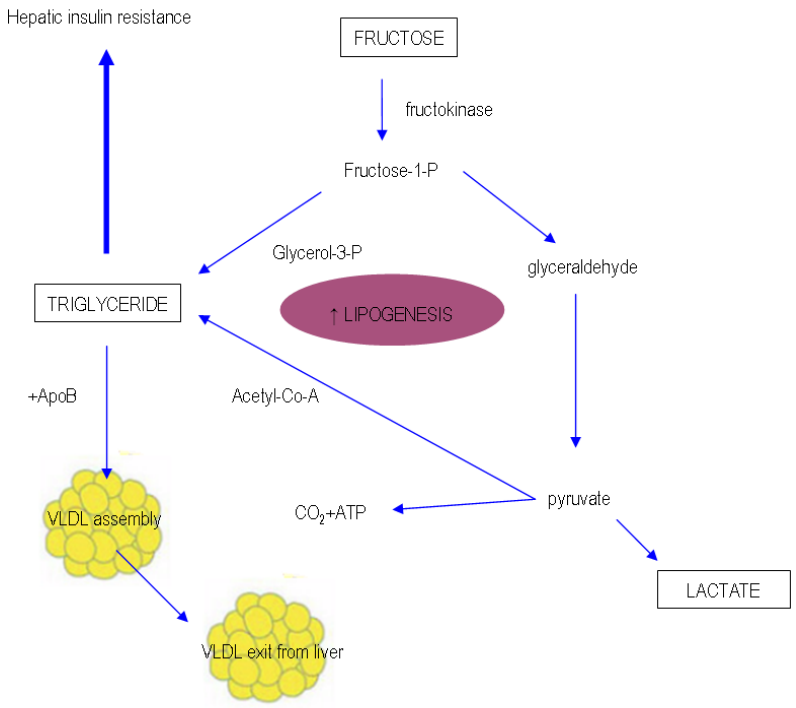

Figure 2.4: Lipogenesis from hepatic fructose metabolism and involvement of ApoB in very low-density lipoprotein (VLDL) assembly and exit from the liver (adapted from [12,31])

When the hepatic lipid concentration increases, the degradation of ApoB is dramatically reduced, and, under conditions of fructose consumption, ApoB concentrations may rise by as much as 25%.[12,31] Several short-term studies have shown fructose to be promoting of unfavourable lipid profiles, with the hypertriglyceridemic effect being more pronounced in the longer term, together with increased postprandial levels of the atherogenic ApoB.[29] Hypertriglyceridaemia is considered an independent risk factor for coronary heart disease, and even moderate increases in VLDL are associated with such changes as reduced HDL and small, dense LDL. The lipoprotein changes are strong components of the MS, and are recognised as risk factors for atherosclerotic disease.[29]

Thus, the postprandial hypertriglyceridemia seen after fructose ingestion is exacerbated in individuals with higher fasting insulin concentrations, suggesting a strong relationship between IR and the lipogenic effects of fructose.[13]

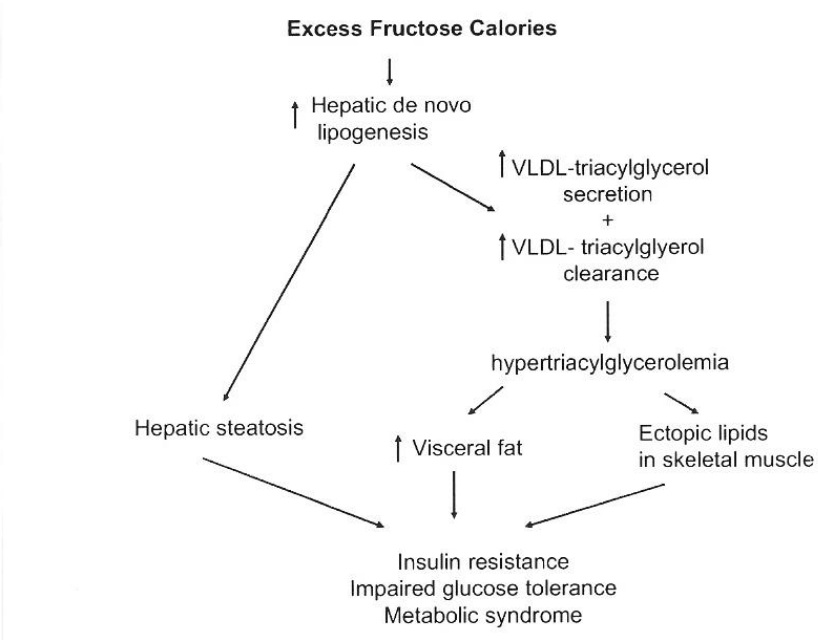

Figure 2.5: The putative mechanisms that link fructose excess to MS[36]

Regardless of the mechanism, it is clear that fructose feeding can induce IR and glucose intolerance. Since the intake of fructose in the American diet has shown to have increased considerably over the past three decades, it is important to examine the effect of fructose on individuals who are predisposed to IR and glucose intolerance, such as those with POS.

Stanhope and Havel investigated the metabolic effects of beverages providing 25% of total calories of energy requirements for 10 weeks, in older, overweight men and women. In their interim report, both patient groups gained an average of 1.5 kg within 8 weeks.[31] As measured by computerised tomography, the fructose group showed a significant increase in intra-abdominal fat, as opposed to the glucose group, which remained unchanged.[31] The researchers concerned report, based on interim results, that a high fructose diet promotes the development of three of the cornerstone characteristics associated with MS, namely:

- dyslipidaemia;
- IR; and
- increased visceral adiposity.[31]

The effect of fructose on diabetic complications is not known.[29] Of interest is the effect of fructose on protein fructosylation and oxidative stress, particularly in diabetic subjects. After consumption of large quantities of fructose, such as juices and sweetened beverages, significant amounts of fructose may resist hepatic uptake.[29] The result is increased circulating fructose concentrations. Since fructose is a major product of the polyol-sorbitol pathway, tissue accumulation of fructose has been implicated in diabetic neuropathy and other diabetic complications.[29] Diabetic neuropathy could reflect increased protein fructosylation, and, in rats, a diet providing 40% of total energy from fructose, increased the formation of cataracts, compared to the incidence of such formation among rats that were fed a high-glucose control diet.[29]

That the regulation of appetite and gastro-intestinal hormones might be dysfunctional in subjects with PCOS is supported by evidence.[6,9,13,29,32,37] Both plasma insulin and leptin act in the CNS in terms of the long-term regulation of energy homeostasis. Because fructose does not stimulate insulin, the consumption of foods and beverages with high fructose content produces smaller postprandial insulin excursions than does consumption of glucose-containing carbohydrate. Leptin production is regulated by means of insulin responses to meals, with a time delay of several hours. A lower insulin response after ingestion of fructose would then be associated with lower serum leptin concentrations than would be the case after ingestion of glucose. Because of the anorexigenic effect of leptin, the lower levels of leptin after fructose intake would serve to enhance appetite and food intake. The combined effects of lowered circulating leptin and insulin in high fructose diets could,

therefore, increase the likelihood of hyperphagia, weight gain and its associated metabolic consequences.[9,13,29]

As in the study of PCOS and the adherence to diet/dropout rate, much attention has been given to the orexigenic gastric peptide, ghrelin, due to its potent effects on stimulating food intake in animals and humans.[37] The researchers concerned showed in their study that the relative elevation of ghrelin after fructose ingestion suggests the failure of fructose to suppress ghrelin, along with reduced insulin and leptin, which could contribute to decreased satiety and to increased food intake during long-term fructose consumption. Glucose-mediated insulin stimulation elevates circulating levels of leptin, and, in turn, the orexigenic gastric peptide, ghrelin, is suppressed.[37]

2.2.4 Summary of literature overview

The current literature study was performed with the goal of highlighting the metabolic and clinical characteristics of both obese females suffering from PCOS and the phenomenon of fructose overload, showing similarities between the two conditions, and posing the question of whether a fructose-overloaded diet might not exacerbate or even bring on the symptoms and condition of PCOS. Several metabolic markers appear in both conditions, separately. Of particular interest is the first metabolic perturbation after fructose consumption, which is hypertriglyceridemia.[31] Predominant risk factors in PCOS for CVD are hypertriglyceridemia and reduced levels of HDL.[4] The mechanisms by which fructose overload cause metabolic aberrations are explained in the foregoing literature study, and the same metabolic and clinical symptoms present themselves in both obese PCOS and fructose overload. The same symptoms that link PCOS to the MS are also present with fructose overload. The symptoms include such clinical measures as increased waistline circumference, elevated BMI, low HDL, and hypertriglyceridemia.

For millennia, the human diet was relatively low in fructose sourced from naturally occurring fructose in fruits and very small amounts of honey.[31] Such a situation implicates that the human diet was designed for a higher hepatic glucose intake, with a relatively small capacity for fructose intake.[31] The sudden, large increase in daily fructose intake, which is mainly metabolised by the liver, disturbs normal hepatic carbohydrate metabolism, resulting in the following two major complications:

- perturbations in glucose metabolism and -uptake pathways; and
- significantly increased DNL and triglyceride synthesis, driven by the high flux of glycerol and acetyl-CoA of triglyceride molecules coming from fructose catabolism.[22]

The above seems to underlie the induction of IR commonly observed in both animal and human models.[31] IR induced by fructose overload is commonly characterised by profound metabolic dyslipidaemia, appearing to result from both hepatic and intestinal overproduction of atherogenic lipoprotein particles. With the knowledge that both fat and fructose have negligible insulin responses, and accepting that the cholesterol, fat and fructose intake of the Western diet is considerable,[31] synergistic actions between the nutrients can readily occur, leading to a greater degree of IR and dyslipidaemia. Shortly, the emerging evidence from epidemiological and biochemical studies indicates that the high dietary intake of fructose has rapidly become a causative factor in the development of the MS. The need for public awareness on the issue in question is urgent. The public should be made aware of the risks associated with high fructose consumption, and, globally, more effort should be made to educate the as yet uninformed public of the dangers of consuming the increased amounts of additional fructose that are used in foods and beverages.

The definition of GI has internationally been established as a postprandial glucose excursion with an insulin response.[4] Since fructose does not fulfil such a definition, the substance

should be excluded from classification by GI. Moran and Norman[15] specifically name fructose as one of the factors that decrease the GI.[15] Fructose is almost fully metabolised by the liver, and, at best, elicits a feeble insulin response,[9,13,29] so that the conclusion can be made that fructose is, in principle, not included in the criteria for GI, and neither should fructose be afforded a GI value. Moran and co-workers[6] noted their concern that, in the literature so far, the utilisation of GI as a strategy for weight loss in obese/overweight females with PCOS has not been shown.[6]

The two sugars, glucose and fructose, should be seen in their different metabolic pathways, and the GI should be afforded as such. Although fruit juice may be partially glucose, it is still misleading to the uninformed public to label a high fructose-containing fruit juice as 'low GI', as the GI value only pertains to the glucose content of the fruit juice. As seen in the literature study, clarified apple and grape juice, predominantly fructose-containing clarified juices (with fructose being sweeter than glucose) is added to increase sweetness, yet fruit juice is still labelled 'pure'.

CHAPTER 3

METHODOLOGY

3.1 AIMS AND OBJECTIVES

The aims and objectives of the current study were as described in the following subsections.

3.1.1 Aim 1: Literature review

A literature review, using PUBMED/MEDLINE was performed to:

- assess the anthropometric and clinical/metabolic profile of the overweight/obese patient presenting with PCOS; and
- describe the metabolic effects of fructose overloading in humans.

3.1.2 Aim 2: Assessment of baseline fructose intake of overweight subjects suffering from PCOS

The baseline fructose intake from edible fruit, sweetened soft drinks and beverages was recorded as accurately as possible, using methodology as described in section 3.3 below.

3.1.3 Aim 3: Assessment of outcomes in terms of the efficacy of fructose restriction

To assess the efficacy of fructose restriction to a maximum of 16–20 g per day,[12] on the improvement in fertility outcome and anthropometric measurements for weight loss, the following outcomes were assessed:

- improvements in anthropometric measurements, namely BMI, WC and WHR (Addendum 1);
- improvement in the biochemical markers of PCOS and MS (Addendum 1);

- improvement of reported subjective clinical symptoms, as documented (Addendum 2a and 2b);
- adherence to guidelines for weight loss, as measured by dropout rate of subjects from the research study,15 over a 1-year period; and
- fertilisation resulting in pregnancy.

For the purposes of the current study, excessive fructose intake was considered as intake of:

- more than 3 servings of edible fruit per day;
- drinking more than 340 ml of any sweetened beverage; or
- more than 200 ml of any fruit juice per day.

3.2 HYPOTHESES

The following null hypotheses were postulated:

- Chronic fructose consumption has no impact on the anthropometric and clinical markers in obese women with PCOS.
- Restriction of fructose will not influence the adherence to a diet designed to bring about weight loss.

3.3 STUDY PLAN

3.3.1 Study design

The study design was that of an experimental cohort.

3.3.2 Study population

The patients were sourced from the Infertility Clinic at Tygerberg Hospital (TBH), and, after being diagnosed according to the Rotterdam consensus statement of 2003 for PCOS,[1]

including a BMI of > 27,[38] were referred for dietary analysis and guidelines/counselling. The population group largely consisted of coloured women residing in the surrounding areas in the Western Cape, and who were unemployed and seeking fertility.

3.3.2.1 Inclusion criteria

The inclusion criteria for the current study were patients:

- diagnosed with PCOS, according to the Rotterdam consensus statement;1
- with a BMI of more than 27;38
- in the age range from 19 to 44 years (which forms the age range set by default in the PUBMED/MEDLINE search); and
- seeking fertility.

3.3.2.2 Exclusion criteria

The exclusion criteria for the current study were patients:

- presenting with any other reason for anovulation or hirsutism;
- with previously diagnosed type 1 or type 2 diabetes;
- who failed to attend any further follow-up consultations after their baseline consultation, and who were therefore viewed as 'defaulters'; and
- who failed to adhere to the advised dietary guidelines, and who failed to show any progress throughout the course of the trial period, thus being viewed as 'defaulters'.

3.3.3 Sample size

For a high-powered study (90% power), as calculated by Prof. D.G. Nel, the statistician assigned to the current study, the population should have included 120 subjects. For reasons described in the Discussion (Chapter 5), 86 patients were baselinely included in the study,

consistent with the inclusion criteria. All the baseline patients could be evaluated to test hypothesis 1. Only 18 patients who participated in 3 visits could effectively be used to test hypothesis 2.

3.4 METHODS OF DATA COLLECTION

3.4.1 Logistical considerations

The physical dietary consultation with patients included in the current research study took place at the Infertility Clinic at Tygerberg Hospital, Bellville, in a small office area, which had previously been dedicated to the weighing and measuring of patients.

3.4.2 Anthropometry

3.4.2.1 Weight

Weight was measured with the baseline assessment of new patients, and with each of their follow-up visits thereafter. The same electronic scale (AE Adam Digital Medical Scale) was used by all parties involved in measuring the actual weight of patients included in the study. The digital scale rounded off the actual weight of the patient to the nearest 0.1 kg. The person overseeing the weighing ensured that all heavy clothing was removed, and that items such as keys, wallets and cell-phones were removed from pockets before the weighing of the patient took place. The patient was asked to wear more or less the same clothing for each visit. Weight was taken using the same procedure upon each visit.

3.4.2.2 Height

Height was measured directly by means of a height-measurement extension, fixed to the AE Adam Digital Medical scale used at the TBH Infertility Clinic. Height measurements were

be rounded off to the nearest 0.01 m[39] and taken without shoes. Height was taken upon the baseline visit only, in order to calculate the BMI of the patient.

3.4.2.3 Body mass index (BMI)

The BMI is the most widely-used index for height and weight, and is a validated measurement for nutritional status.[39] The metric formula for the calculation of BMI is:

$$\text{Weight (kg)} \div \text{Height (m)}^2 \qquad \ldots(1.1)$$

The index that is popularly known as the BMI was previously known as 'Quetelet's index', being named after the founder of the equation, and the term 'BMI' was popularised, based on the Quetelet Index.[40] Many investigators consider the index concerned to be the best index of body mass in adult population groups, as it is not biased by height, and it is easy to calculate. Since 1984, a Quetelet's index of 25 was considered overweight, and > 30, obese. Health and Welfare Canada, set up tables for optimal BMI with regards to health risk, in terms of which the range of BMI 20–25 is termed 'ideal'. In general, a BMI above 27 kg/m^2 indicates obesity and increased risk for health problems.[40] The range of BMI between 25–27 is termed as "may be associated with health problems for some people".[40]

In Siebert et al.'s (2009) study,[38] the range of BMI associated with a better response to drug treatment for ovulation induction, was 27–35. For the purposes of the current study, a BMI of above 27 kg/m^2 was considered obese,[38,39] and, since one of the aims of the study included fertilisation during pregnancy, the goal BMI for patients before undergoing ovulation induction would be 27–35 kg/m^2.

3.4.2.4 Waist circumference (WC)

The WC was measured using a non-stretchable tape measure, around the smallest area below the rib cage, and above the umbilicus.[39] Measurements were taken in centimetres and rounded off to the nearest 0.1 cm. The WC circumference was measured at the baseline visit, and with every follow-up visit thereafter.

For ideal WC measurement, patients should fast overnight prior to the measurement and wear clothing conducive to the tape being placed in the correct positioning. The space between the lowest rib margin and the top of the iliac crest should be palpated and marked with a felt-tip pen. The space midway between the iliac crest and the lowest rib margin should be measured by means of a non-stretchable fibreglass tape. Patients should be asked to breathe normally, and should not contract their stomach muscles or hold their breath. The reading should be taken to the nearest millimetre.[40]

The waist–hip circumference ratio is a simple method that is used for describing both subcutaneous and visceral adipose tissue.[40] A WC of > 88 cm in females is described as a diagnostic factor in MS.[10]

3.4.2.5 Hip circumference

The subject should stand erect, not uplifting arms, but keeping arms at the sides, with feet together. The measurement was taken over the maximum extension of the buttocks, with the tape touching the skin, but not indenting the soft tissue.[40] Although the hip circumference alone had no reference value, the measurement was taken in order to calculate the WHR.

3.4.2.6 Waist–hip circumference ratio (WHR)

In addition to the BMI, some measure representing subcutaneous fat should also be included in the assessment of leanness or obesity.[40] The waist–to–hip ratio (WC in cm ÷ hip

circumference in cm) has been recommended as a measure of fat distribution, for use in conjunction with the BMI (Quetelet's index).[40] The suggestion was made in 1985 that WHRs for men of > 1.0 and > 0.8 for women were indicative of cardiovascular complications and related death.[40] More recent publications mention that a WHR of > 0.85 is common amongst overweight patients suffering from PCOS.[5] The WHR was calculated by the statistician assigned to the current study protocol, from the values supplied as WC and hip circumference.

3.4.3 Standardisation

The researcher alone took all measurements of WC. With regards to weight and height, in order to establish BMI at the baseline visit, the researcher provided the necessary training to the nursing staff involved with the measurements concerned. As far as possible, only one member of the nursing staff (in addition to the researcher) was given responsibility for the taking of height and weight, in order to ensure maximal standardisation of the procedures involved.

3.4.4 Subjective clinical symptoms

Subjective clinical symptoms,[3] were assessed in a detailed 'yes'/'no' questionnaire (Addenda 2a and 2b), with changes in the perception of the patient as to whether the symptoms experienced had improved or not being recorded at each visit. At each follow-up visit, the patient filled out another questionnaire, without being provided with any further insight into the answers that were given on the previous visit.

3.5 DIETARY COUNSELLING

3.5.1 Counselling

The researcher attended the Infertility Clinic every Monday morning from 09h00–13h00 between beginning November 2009 and end of April 2011. Each patient was afforded an baseline consultation of at least one hour in length. The researcher started the consultation with an in-depth history-taking, assessing a 24-hour recall, asking about general habits, favourite foods, and the environment in which meals were eaten (Addendum 3). Detailed questions were asked about the consumption of drinks and beverages, and emphasis was placed on recalling the amounts and brand names of the beverages consumed. A cross-check was done after the baseline questioning, to ensure that the information received from the patient was as accurate as possible.

In Addendum 4, the specific areas of dietary education given to the patient after history-taking, are described. The main areas of emphasis were to motivate the patient to remove all items of poor food choice, such as sweets, chocolates and crisps, from their diet. The patients were also encouraged to eat three meals, and snacking was discouraged. The protein proportion of the meals was explained using a drawing of a plate, indicating the composition of the meal with regards to protein, starch, and vegetables. In order to reduce the GL of the diet, the patients were allowed to include starch in their breakfast and lunch meals, but had to omit starch from the supper meal, in favour of a larger intake of vegetables than they would otherwise have had. Lastly, the patients were strongly discouraged from taking any sweetened beverages, and encouraged rather to eat their fruit in whole fresh form, with preferably no more than three servings of fruit daily.[12] They were encouraged to replace their drinks with water (still or sparkling) or diet-type drinks.

At the outset of the study protocol (in 2009), a website (www.thepaleodiet.com/index.shmtl)[41] was accessed to obtain a table giving the fructose content of various fruits and dried fruit, which was useful in calculating fructose intake from fruit. However, by the end of the study the website table no longer existed. During dietary history-taking, patients were pertinently asked about the exact brand names of the sweetened beverages and types of fruit juice and fruit that they consumed. The patients were asked to recall the amounts of drinks, juices and fruit consumed daily, as accurately as possible. Differences in intake over weekends were also taken into account, and daily intake adjusted to reflect an average daily intake.

The percentage of carbohydrate from each mentioned drink was obtained from the label, stated as an amount (in g) per 100 ml of the drink. The list of ingredients on the label named the sweetening agent, and, depending on the order of listing of such, the percentage of fructose could be calculated. For example, if a fizzy drink contained 12 g carbohydrate per 100 ml, and the sweetening agent used was sucrose (50% glucose + 50% fructose), the intake of fructose was 6 g per 100 ml. If the sweetening agent was sucrose, listed first, and fructose listed second, the researcher took the percentage of fructose as being 70% of the total carbohydrate content. Fruit juices posed a slightly bigger problem, as they vary widely in the amount of fructose that they contain, depending on whether they are juice-sweetened, or whether they are sweetened through pure, crystalline fructose being added as a 'natural' sweetener. The fructose content of the whole fresh fruit, as per the mentioned table of fructose content of fruits, was also used to guide the researcher to draw conclusions about the percentage of fructose in juices. Generally, apple, grape, mango and pear juices, by nature, have the highest contents of fructose, and the researcher assigned a value of 75% of total carbohydrate as fructose to the juices concerned. Juice such as orange juice and juices that

were not sweetened with clarified apple or grape juices were assigned a fructose content of 40–45% of the total carbohydrate content.[29]

3.5.2 Follow-up

The form, as set out in Addendum 5, was used to monitor the progress of the patients with each follow-up visit. Pertinent questions were asked to ascertain the patient's compliance with fructose restriction, as well as with the guidelines for weight loss. The follow-up was conducted only by the researcher. The patients were followed up on a 3-monthly basis. Each visit included re-evaluation of the following:

- patient weight, BMI and WC (Addendum 1);
- biochemical markers of PCOS and MS (Addendum 1);
- completion of the questionnaire on subjective clinical symptoms (Addendum 2);
- 24-hour dietary recall, including discussion of problems that the patient might have had in adhering to the prescribed dietary guidelines (Addendum 5); and
- a quick summary and reinforcement of the dietary guidelines, as well as encouragement to continue.

The process remained the same for each 3-monthly follow-up visit during the 1-year period.

3.6 ANALYSIS OF DATA

MS Excel was used for capturing the data and STATISTICA version 8 and the Statistical Application System (SAS) were used to analyse the data. Summary statistics were used to describe the variables concerned. Distributions of variables are presented using histograms and/or frequency tables. Medians or means were used as the measures of central location for ordinal and continuous responses, and standard deviations and quartiles as indicators of spread.

Relationships between two continuous variables were analysed with regression analysis. The strength of the relationship was measured with the Pearson or Spearman correlation if the continuous variables were not normally distributed.

The relationships between continuous response variables and nominal input variables were analysed using appropriate analysis of variance (ANOVA). When repeated measures on a response variable were compared against a nominal input variable, a repeated-measures ANOVA (RMANOVA) was used with the compound symmetry option to determine the correlation structure over time. The analyses conducted, which were also done using STATISTICA, were confirmed with PROC MIXED in SAS. SAS's PROC MIXED was also used when the influence of an external covariate required taking into account in RMANOVA.

If the RMANOVA test indicated significant differences among the mean responses (i.e. if $p<0.05$), then a Bonferroni multiple comparisons analysis was done to detect where the differences occurred.

A ρ-value of $\rho < 0.05$ represents statistical significance in hypothesis testing, and 95% confidence intervals were used to describe the estimation of unknown parameters.

A repeated-measures ANOVA (RMANOVA) test was done on BMI, with fructose as covariate. The analysis was done in SAS with PROC MIXED. The assumption of compound symmetry was used as the correlation structure of observations on the same subject over the times observed. The result indicated that, in the current study population, fructose was a covariate to BMI ($\rho=0.006$).

3.7 ETHICAL CONSIDERATIONS

3.7.1 Informed consent

The researcher provided all patients with an informed consent form, as is presented in Addendum 6, and which was translated into the language of the patient's choice. Only English- and Afrikaans-speaking patients were encountered, and it was not found necessary to employ an isiXhosa-speaking interpreter. The consent form was an adapted version of the standard informed consent form that is used by the Faculty of Health Sciences at Stellenbosch University.

3.7.1.1 Ethics Review Committee

The study was submitted for approval to the Health Research Ethics Committee, Faculty of Health Sciences at Stellenbosch University. and approval was given on 06 November 2009, Ethics Ref. No.: **N09/07/181.**

3.7.1.2 Patient confidentiality

Patient identification information was omitted from study-related material to ensure participant confidentiality. Upon entering the study, each participant received a subject identification number that was used on all study-related material and documentation.

The participant was assured both verbally and by means of the informed consent form that all conversation and information provided to the researcher would be regarded as confidential.

Information provided to the researcher was only to be used for the specified study, and was not to be shared for any other purposes or studies.

3.8 BUDGET

The expenses for the current study were baselinely carried by the researcher, with the patients not being charged for baseline consultations and follow-up visits. The intervention thus cost nothing more to the patient other than what they would routinely have been charged for receiving infertility treatment at Tygerberg Hospital. In 2010, the researcher was granted an assistant research post by the Department of Obstetrics and Gynaecology, Stellenbosch University, to the amount of R16 000.00 (inclusive of PAYE). Table 3.2 below details the description of expenses and the approximate cost of the current study.

Table 3.1: Description of expenses and approximate cost of study

Description of expenses	Approximate cost
Travelling costs to TBH	R2 000.00
Loss of income due to non-attendance of private practice	R65 500.00
Stationery and printing	R2 000.00
Telephone	R2 000.00
University fees	R26 000.00
Assistant Research Post (less PAYE)	R32 000.00 (credit)
Total cost to researcher	**R65 500.00**

3.9 TIME SCHEDULE

Table 3.2 below gives the time schedule for the current study.

Table 3.2: Time schedule for the current study

Aspect of research	Approximate length of time needed (months)	Estimated date
Preparation of protocol	2	End October 2009
Approval by Ethics Committee	2	Mid-November 2009
Data collection	18	November 2009 – April 2011
Data analysis	2	End June 2011
Oral report of results at Academic Year Day	2	17 August 2011
Final adjustment / Additional time	12	August 2012

In the following chapter (Chapter 4), a detailed discussion regarding the interpretation of the results of the research study, including the information gathered during the course of the literature study, follows.

CHAPTER 4

RESULTS

4.1 INTRODUCTION

The subjects for the current study were recruited from the Infertility Clinic at Tygerberg Hospital, Bellville, Cape Town. After being diagnosed with PCOS,[1] the subjects were referred for dietary intervention. All dietary consultations were done free of charge. The study baselinely recruited 86 subjects, who complied with the inclusion criteria.

The baseline profile of the study population is described, followed by assessment criteria for the efficacy of fructose restriction over the 18-month course of the study:

- changes in anthropometric measurements (Addendum 1);
- changes in biochemical parameters (Addendum 1);
- improvements in clinical symptoms and dietary practices and behaviours (Addendum 2);
- fertilisation, resulting in pregnancy; and
- the dropout rate of subjects from the study.

A. BASELINE DATA

4.2 POPULATION PROFILE

The mean age of the study population was 28.5 ± 4.2 years. Most of the subjects were between 25 and 30 years old, and the oldest subject included was 38 years old (Figure 4.1).

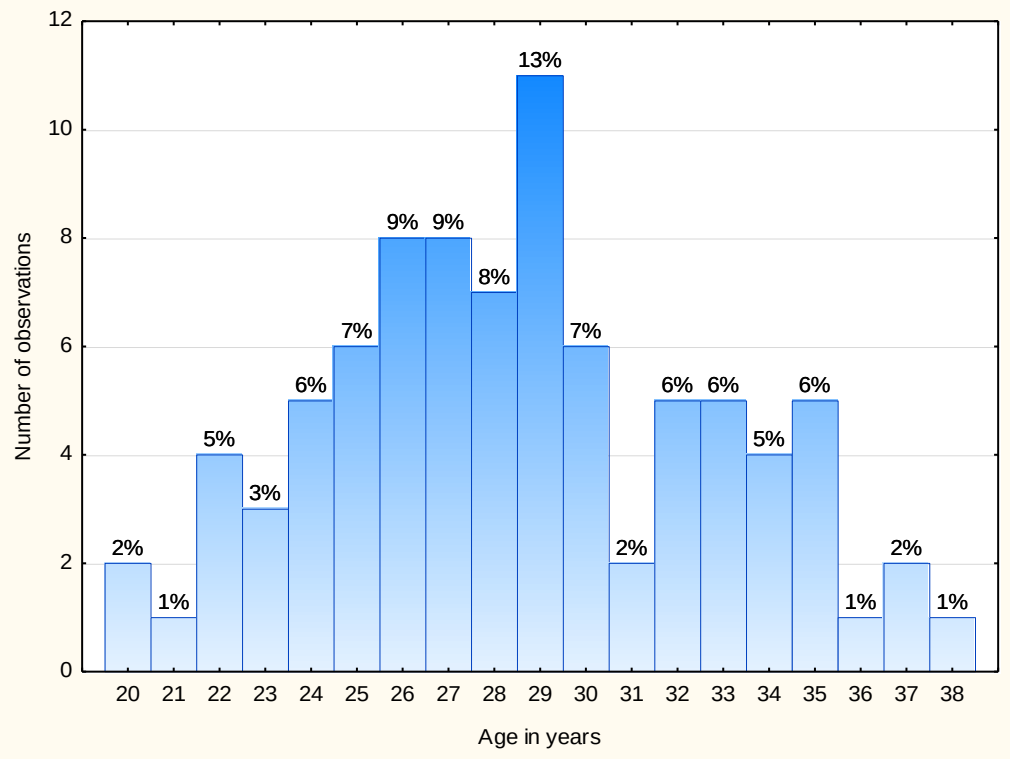

Figure 4.1: Age of the study population

No data were kept as to the number of pregnancies, live children or number of previous visits undertaken to the infertility clinic. The subjects were encouraged to show proof of employment, and discouraged from using infertility treatment if they and/or their spouse/partner were unemployed.

4.2.1 Anthropometric measurements

4.2.1.1 Weight

The average weight of the subjects was 99.8 ± 24.8 kg, and half of the subjects weighed between 80 and 100 kg (Figure 4.2). The highest weight recorded was 240 kg (Figure 4.2).

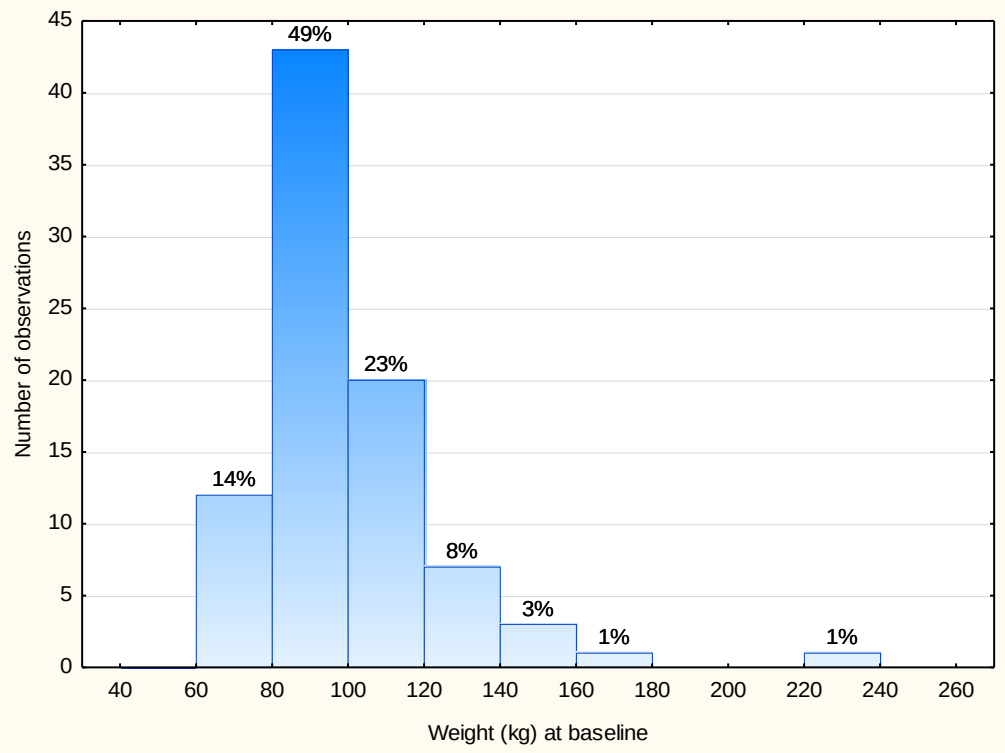

Figure 4.2: Weight (kg), upon baseline visit, of study population

4.2.1.2 Body mass index (BMI)

The mean BMI of subjects assessed was 39.2 ± 8.7 kg/m^2. Of the subjects, 95% presented with a BMI of above 30 (Figure 4.3), with the remaining 5% being overweight. The highest BMIs recorded were 67 and 85 kg/m^2.

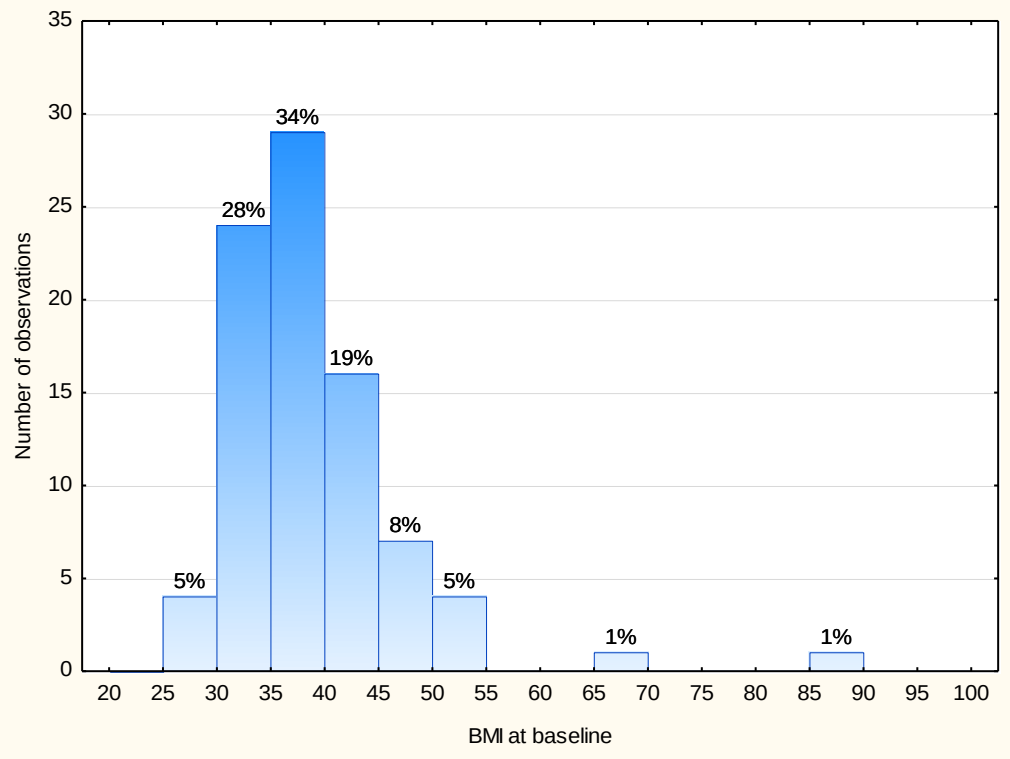

Figure 4.3: BMI of study population on baseline visit

4.2.1.3 Waist circumference (WC) and waist–hip ratio (WHR)

The average WC of subjects was 107.3 ± 11.5 cm, ranging from 84 cm to 135 cm. Of the baseline subject population, 5 subjects had a WC of less than 90 cm, and the smallest WC measured was 80 cm.

The mean WHR was 0.89 ± 0.07, with the documented cut-off for healthy females being suggested as no more than 0.85.[40] In the current study, 75 women (87%) exceeded the value concerned (Figure 4.4).

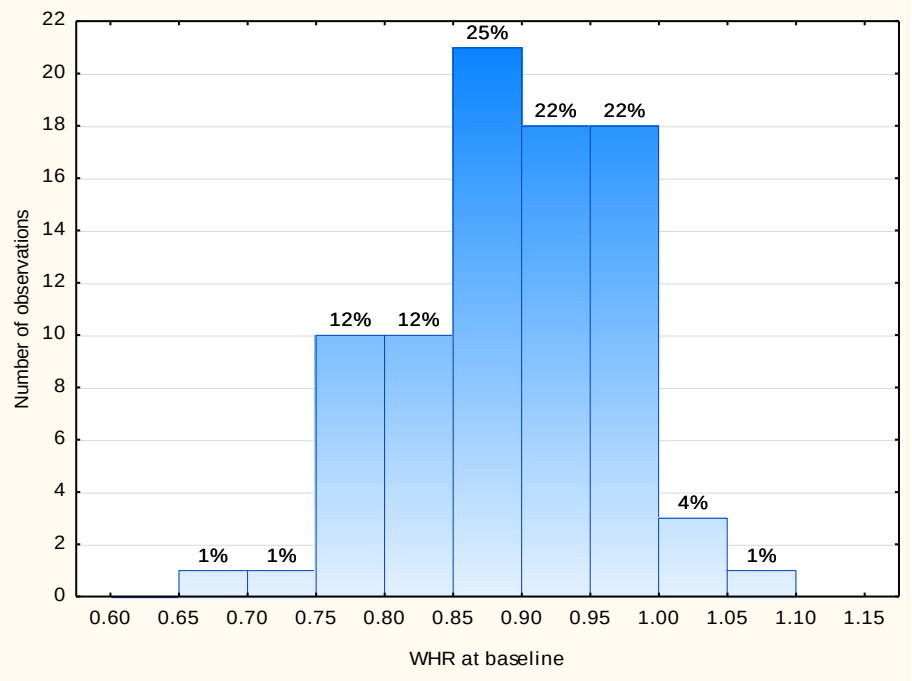

Figure 4.4: Waist–hip ratio of study population on baseline visit

From the baseline study population (n=86), 73 subjects presented with an 'apron effect', which meant that excessive fat from the lower abdominal area was included in the hip measurement. In the rest of the subjects (n=13), the same effect was not present.

4.2.2 Biochemical measurements

Due to reasons beyond the influence of the researcher, it was decided not to perform biochemical parameters on patients included in the study. Since no results were obtained in such respect, no further mention is made of biochemical parameters in the monitoring of the progress of the study population concerned.

4.2.3 Clinical symptoms and dietary practices/behaviours

The clinical symptoms chosen for the current study include those described in the literature[3] and were indicated on a 'yes'/'no' basis.

4.2.3.1 Clinical symptoms

Clinical symptoms form an important part of the holistic symptomatic picture of the PCOS patient.[3] The symptoms were taken from the literature[3] and frequently reported symptoms, as per the experience of the researcher, were included in the questionnaire.

Table 4.1: Number of participants answering 'yes' to questions regarding clinical symptoms

Clinical symptom	Number (N)	% subjects answering 'yes'
Burning feet	34	61
Cravings	69	79
Symptoms of irritable bowel	71	82
Fatigue	60	69
Hypoglycaemia	43	49
Frequent hunger	50	57
Mood swings	62	71
Frequent waking	52	60

Except for hypoglycaemia, more than 50% of subjects reported 'yes' to the questions regarding clinical symptoms.

4.2.3.2 Dietary practices and behaviours

Table 4.2: Number of 'yes' answers received to questions regarding dietary practices and behaviours

Dietary practices and behaviours	(N)	% subjects answering 'yes'
Night eating	25	29
Daily take-away meals	74	91
Daily unhealthy snacking	81	99
Regular late-evening snacking	67	85

Daily take-away meals, daily unhealthy snacking and regular late-evening snacking were reported by 85% and more of patients. Almost all patients (99%) reported consuming

unhealthy snacks on a daily basis, and 91% reported eating daily meals from fast-food outlets.

4.2.3.3 Previous dietary success

Additional symptoms, dietary practices and behaviours that the researcher had encountered through experience, as being under-reported or frequently reported but under-recorded, were included in the questionnaire. Amongst others, the previous dietary success of subjects and the baseline daily fructose intake were included.

Of the 86 subjects baselinely recruited, 46 (53%) had been referred for dietary counselling to a registered dietician, in order to lose weight. Only one subject reported having had any success.

Of the subjects, 58% (50 cases) reported having attempted to lose weight on their own, with no success.

4.2.3.4 Fructose consumption

The fructose intake was calculated from reported intakes of fruit juice and other sweetened soft beverages, and dried and fresh fruit only (Chapter 3). The baseline average fructose intake of the study population was 167.4 ± 116.8 g. Of the subjects, 61% (n=47) of those who were baselinely assessed reported daily intakes of fructose of more than 100 g / day (Figure 4.5). A fructose intake of 50 g or less was noted by 13% (n=11). Fructose intakes of 450 and 600 g / day, respectively, were reported by only two subjects.

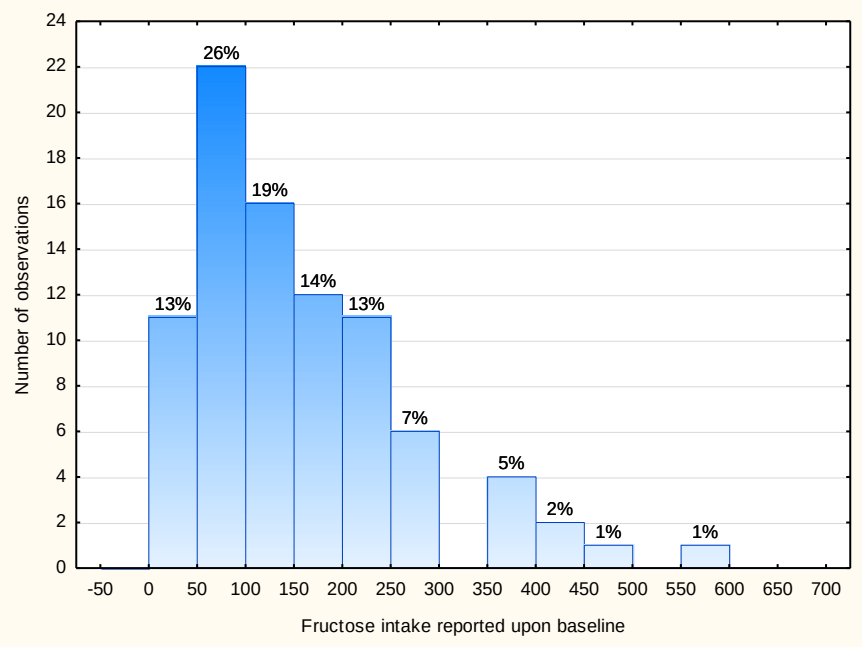

Figure 4.5: Approximate daily fructose consumption (g) of study population, as reported at baseline

4.3 RELATIONSHIP BETWEEN VARIABLES AT BASELINE

4.3.1 Relationship between anthropometric measurements and clinical symptoms / dietary behaviours at baseline (n=86)

4.3.1.1 Body mass index (BMI)

If the residuals from an ANOVA test were not normally distributed, the ANOVA was repeated, non-parametrically, with the Mann-Whitney (MW) test to confirm the ANOVA results. The p- values reported below are for the original parametric ANOVA.

Table 4.3 indicates the relationships between baseline BMI and various clinical symptoms measured. No significant relationships were found between BMI and clinical symptoms.

Table 4.3: Relationship between baseline BMI and the clinical symptoms of study participants, as reported (n=86)

Symptom	BMI value for 'yes' group (kg/m^2)	BMI value for 'no' group ($kg/m^{2)}$	ρ-value (MW)
Cravings	39.4	38.4	0.68
Fatigue	38.5	40.6	0.31
Night eating	39.4	38.7	0.74
Irritable bowel	38.9	40.3	0.56
Mood swings	39.7	37.8	0.38
Snoring	40.2	37.5	0.15
Frequent waking	37.9	41.0	0.11
Daily unhealthy snacking	39.2	49.3	0.26
Daily take-away / fast-food meals	39.5	37.6	0.59
Regular late-evening snacking	39.8	36.7	0.29

4.3.1.2 Waist circumference (WC)

No statistically significant relationships were found between WC at baseline and burning feet, cravings, fatigue, night eating, symptoms of IBS, and perception of mood swings. A statistically significant relationship was found between the baseline WC and the reporting of snoring (Figure 4.6), where a higher WC (mean = 109.5 cm) was significantly associated with the presence of snoring. The subjects reporting "no" to snoring had a mean WC of 103.9 cm.

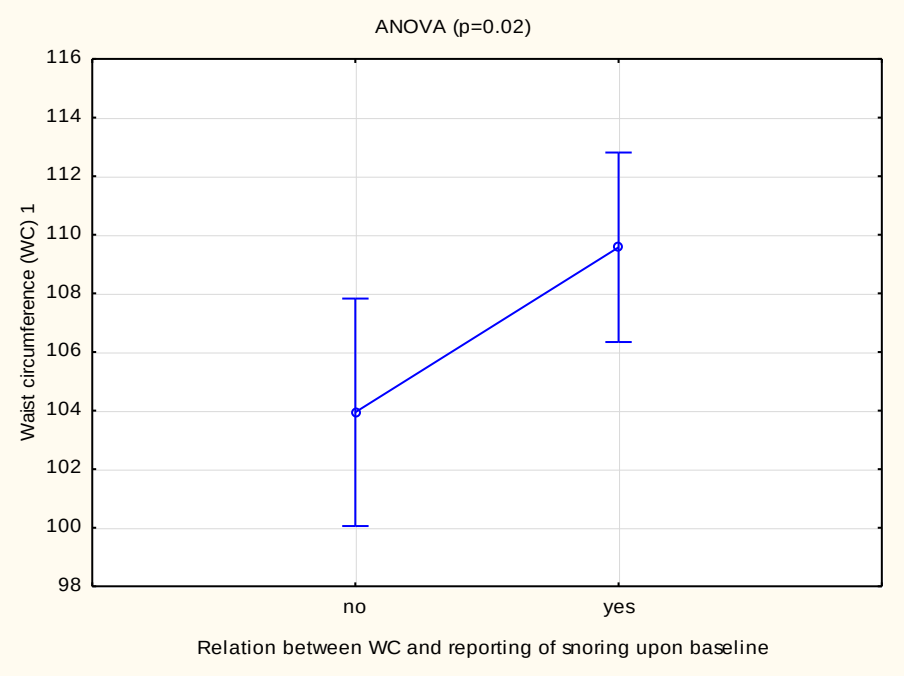

Figure 4.6: Relationship between baseline WC of and snoring in the study population (n=86)

A trend was noted (ρ=0.06), between baseline higher WC and the daily intake of meals from take-away or fast-food outlets (Figure 4.7). The mean WC of the subjects answering "yes" was 108.1 cm, and the mean for subjects answering "no" was 99.5 cm.

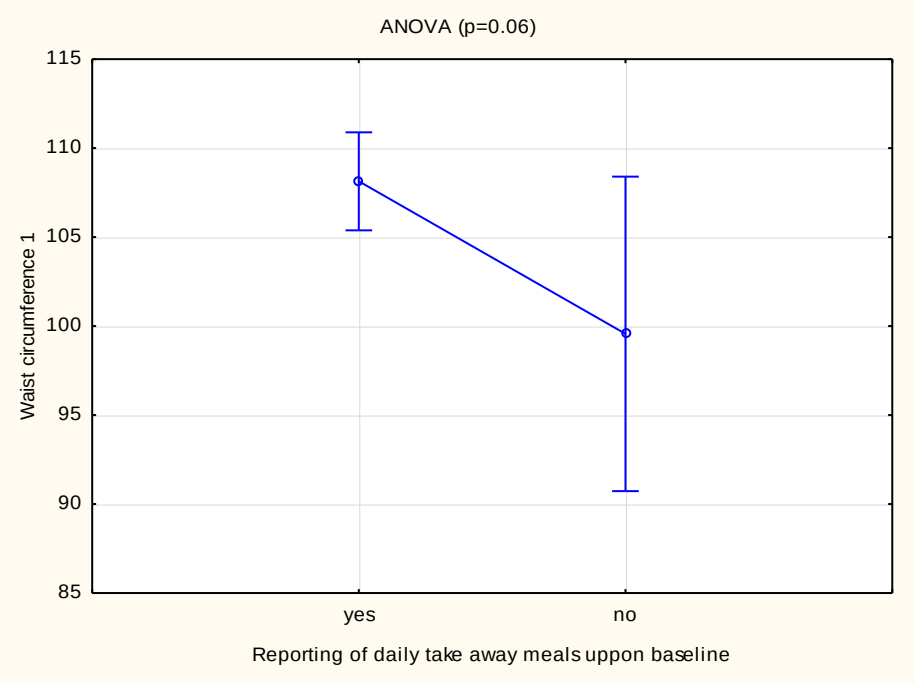

Figure 4.7: Relationship between baseline WC of, and reporting of daily take-away meals by study population (n=86)

No other statistically significant relationships could be found between baseline WC and reporting of frequent waking, regular unhealthy snacking and late-evening snacking.

4.3.1.3 Waist–hip ratio (WHR)

No statistically significant relationships were found between baseline WHR of patients and burning feet, cravings, night eating, symptoms of irritable bowel, or perception of mood swings. A significant relation was shown between baseline higher WHR and reporting of urgent hunger (Figure 4.8). The mean WHR for subjects reporting "yes" was 0.92, while for those reporting "no" the mean WHR was 0.88.

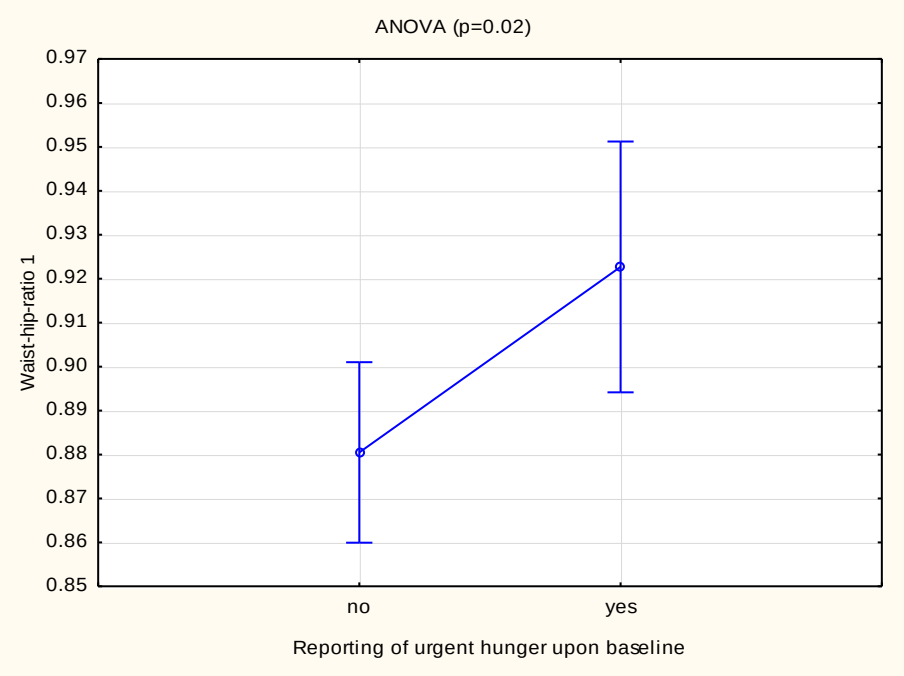

Figure 4.8: Relationship between baseline WHR of, and reporting of urgent hunger by, study population (n=86)

4.3.2 Relationship between baseline fructose intake and baseline anthropometric measurements

The baseline fructose intake of the study population (n=86) was tested against all baseline anthropometric measurements (weight, WC, WHR and BMI), including age. A statistically significant negative correlation was found in the correlation between baseline fructose intake and weight (ρ=0.03) (Figure 4.9) (Spearman Rank test). The contribution to variance in weight was 5.06%, and although significant, the correlation was not strong. Baseline fructose intake did not significantly correlate to any of the other anthropometric measurements.

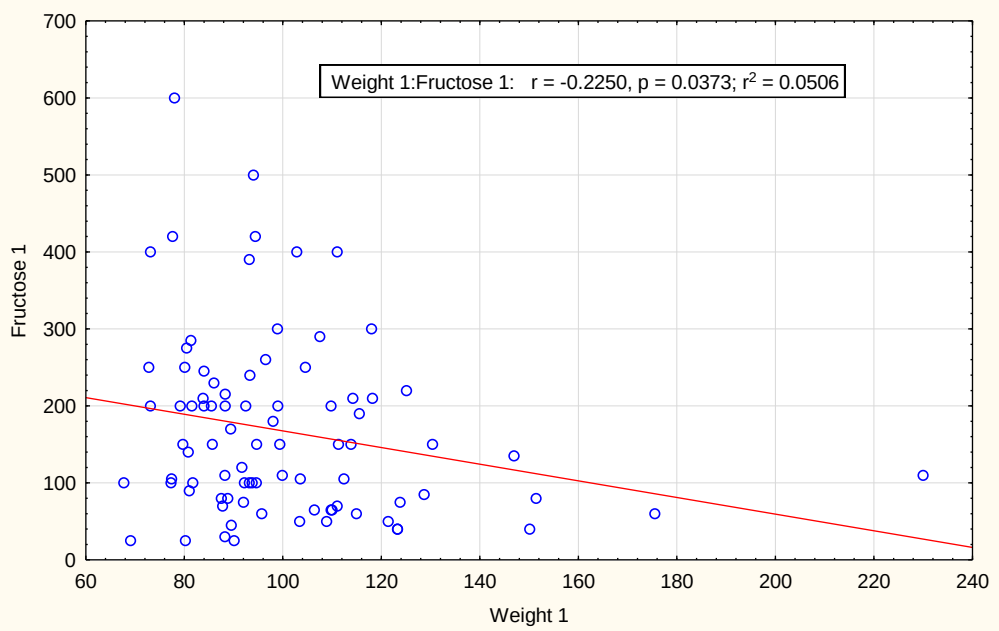

Figure 4.9: Correlation between baseline fructose intake and weight of study population (n=86)

4.3.3 Relationship between baseline fructose intake and reporting of clinical symptoms/dietary practices

Statistical significance (ρ=0.02) was shown between baseline fructose intakes and subjects reporting burning feet (Figure 4.10). Those subjects reporting "yes" had a mean fructose intake of 184.7 g, and those reporting "no" had a mean fructose intake of 119.09 g.

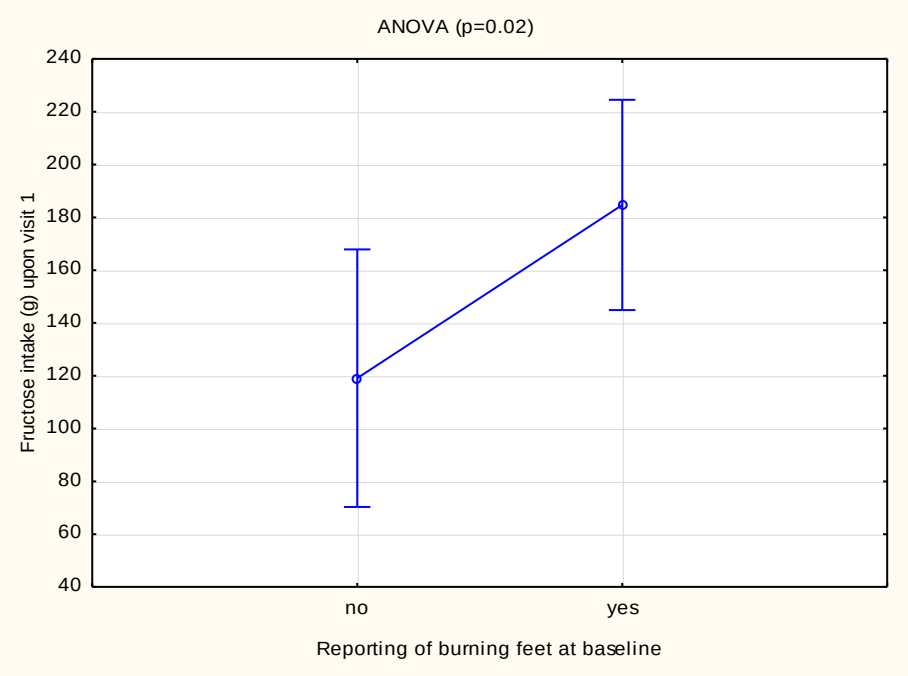

Figure 4.10: Reporting of burning feet in relation to fructose intake among study population on baseline visit (n=86)

The results of the current study showed no significant relation between the reporting of cravings and baseline fructose intake (Figure 4.11). The subjects answering "yes" to cravings had a mean fructose intake of 166.6 g, while the subjects answering "no" to cravings, had a mean fructose intake of 170.5 g.

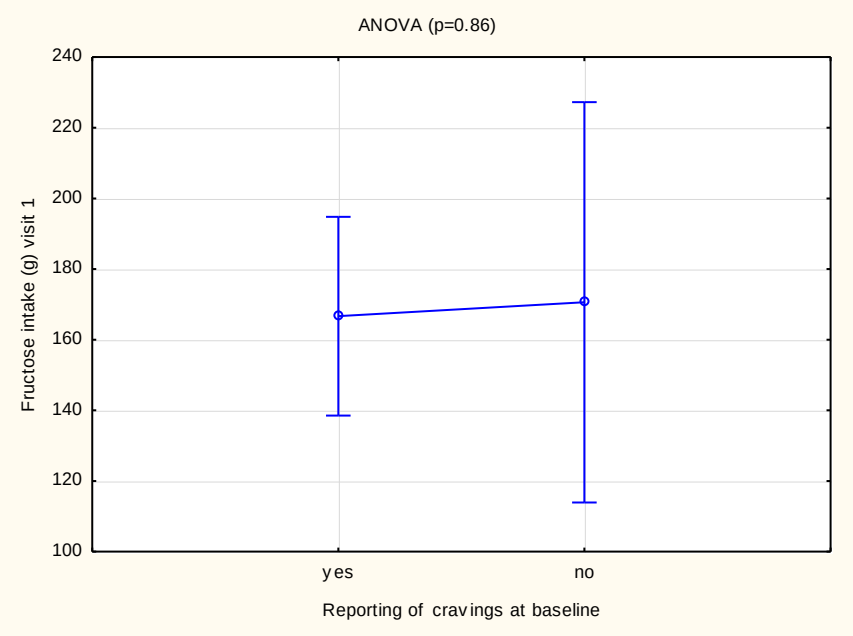

Figure 4.11: Relationship between baseline fructose intake and reporting of cravings by study population (n=86)

A relationship existed between the baseline intake of fructose and the reporting of night eating (ρ=0.07) (Figure 4.12), although the relationship concerned did not reach statistical significance. For the subjects reporting "no", the mean fructose intake was 154.5 g, and for the subjects reporting "yes" the mean fructose intake was 199.0 g.

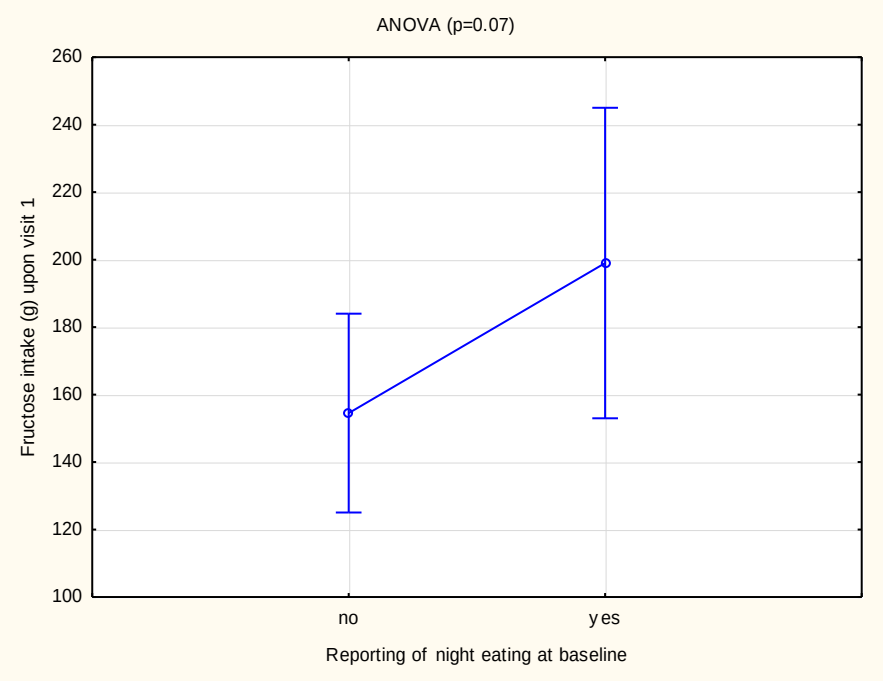

Figure 4.12: Relationship between baseline fructose intake and reporting of night eating by study population (n=86)

A significant (ρ=0.02) relationship was shown between fructose intakes and the reporting of frequent waking upon the first visit (Figure 4.13), with those answering 'yes' showing a mean fructose intake of 190.3 g, and those answering "no" a mean fructose intake of 132.5 g.

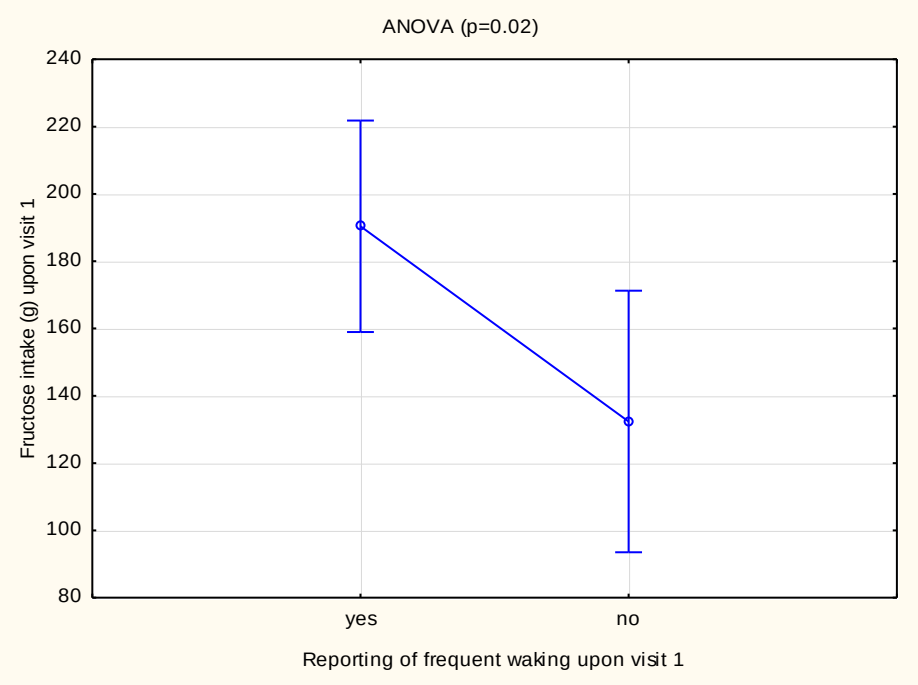

Figure 4.13: Fructose intake in relation to reporting of frequent waking by study population at baseline

A highly significant relationship (ρ=0.01) was found between the reporting of daily take-away meals from fast-food outlets and fructose consumption (Figure 4.14). The subjects answering "yes" to daily consumption of take away meals had a mean fructose intake of 174.3 g, while those answering "no" had a fructose intake of 76.4 g.

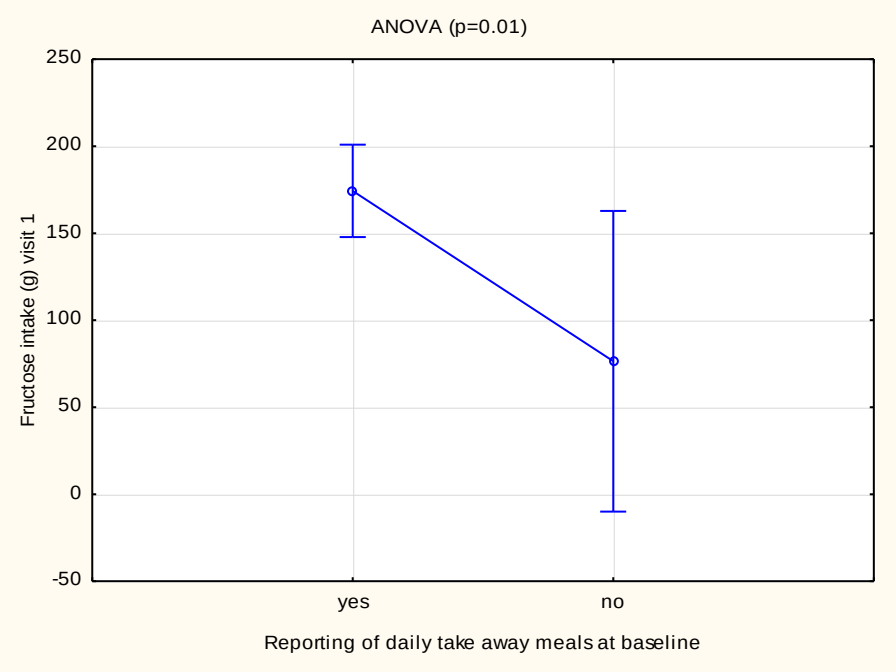

Figure 4.14: Relationship between baseline fructose intake and daily consumption of take-away meals from fast-food outlets by study population (n=86)

No other statistical relationships were found between baseline fructose intake and reporting of fatigue, frequent or urgent hunger, perception of mood swings, snoring, regular unhealthy snacking, or late-evening snacking.

B. FOLLOW-UP VISITS

4.4 DROPOUT RATE

After baseline assessment and consultation of 86 subjects, only 43 returned for a three-month dietary follow-up, constituting a dropout rate of 50% (Figure 4.15).

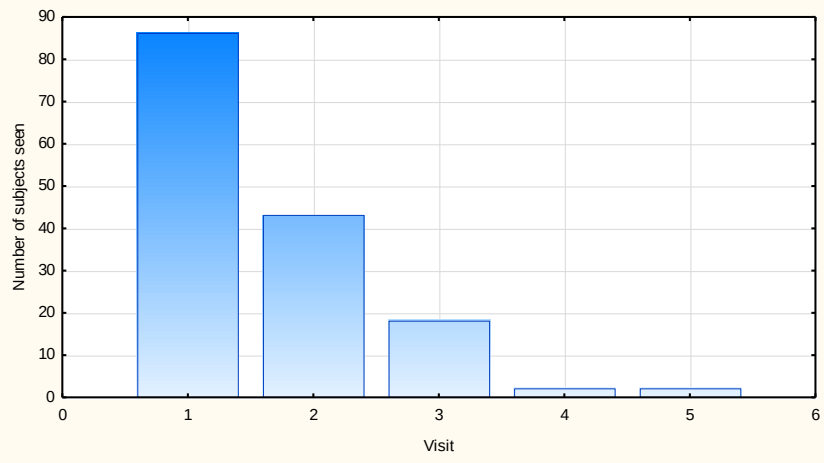

Figure 4.15: Dropout of subjects from baseline dietary consultation

Of the 43 subjects that came for the first follow-up visit, only 18 returned for a third visit, constituting a further dropout of 41% of the first follow-up group. Only 2 subjects returned for a fourth and fifth visit. Statistically, only the 18 subjects (n=18) who had 3 visits for dietary management could effectively be included for evaluation in the current study.

Although the dropout from the first visit was extremely high, the baseline 86 patients assessed proved to be of valuable use in their assessment, as is discussed in Chapter 5.

4.5 CHANGES IN DIFFERENT PARAMETERS

4.5.1 Changes in anthropometrical markers (weight, WC, WHR and BMI)

Weight loss had not reached statistical significance after two visits for dietary counselling (n=43) (Figure 4.16), although it tended to decrease. The mean weight for the baseline assessment (n=86) was 104.36 kg, and upon the second visit was 100.2 kg (n=43).

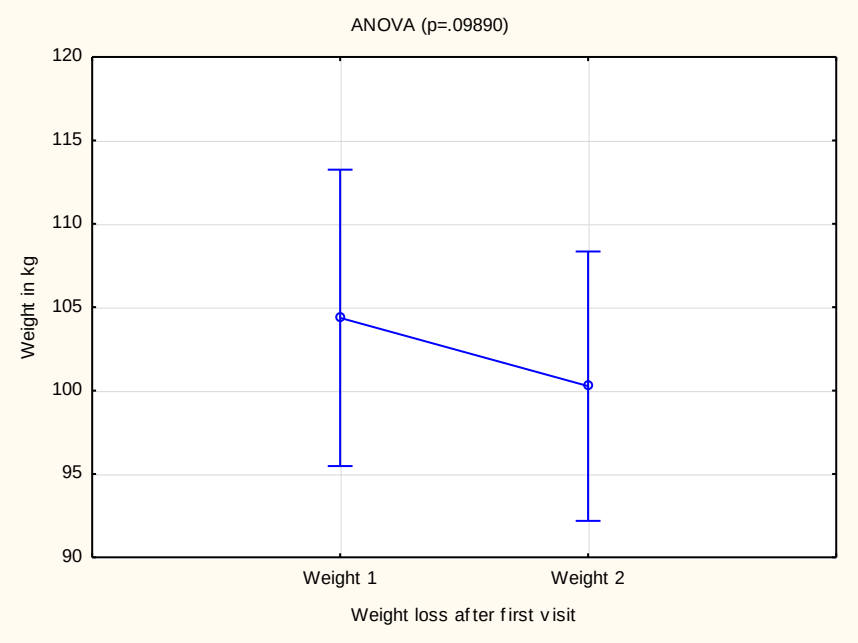

Figure 4.16: Weight loss noted with second dietary consultation

However, after three consultations (n=18), the weight loss of the subjects reached statistical significance, as per Figure 4.17. A Bonferroni multiple comparisons test showed that weight 1 (106.7 kg) differed significantly from weight 3 (104.7 kg).

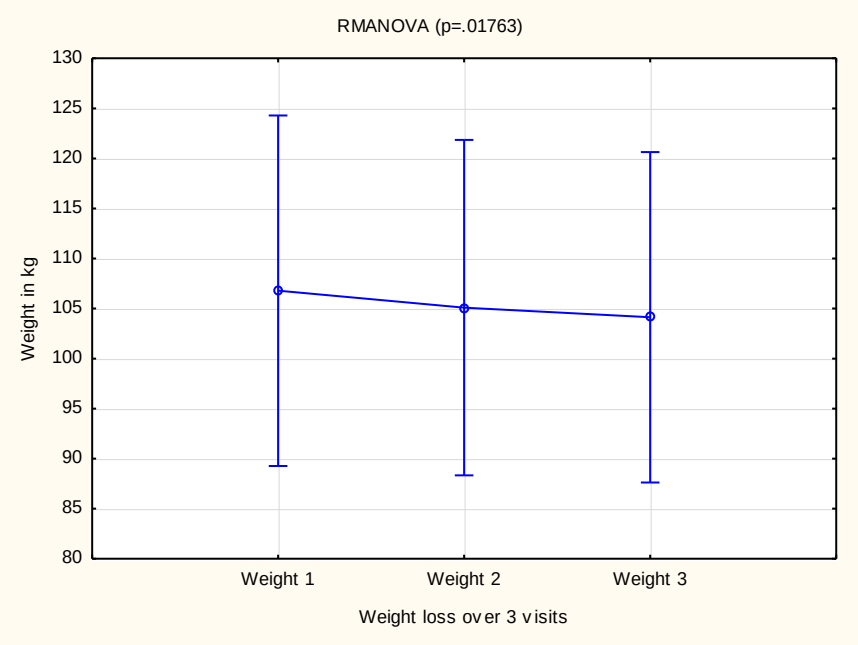

Figure 4.17: Weight loss of subjects over three dietary consultations (n=18)

After 3 dietary consultations, neither the WC (ρ=0.3) nor the WHR (ρ=0.9) of subjects showed statistically significant change, although the general trend was downwards.

However, the BMI of the remaining 18 subjects showed a statistically significant reduction of 1.1 kg/m^2 (ρ=0.019) (Figure 4.18). A Bonferroni multiple comparisons test showed that BMI 1 (41.7 kg/m^2) differed significantly from BMI 3 (40.6 kg/m^2).

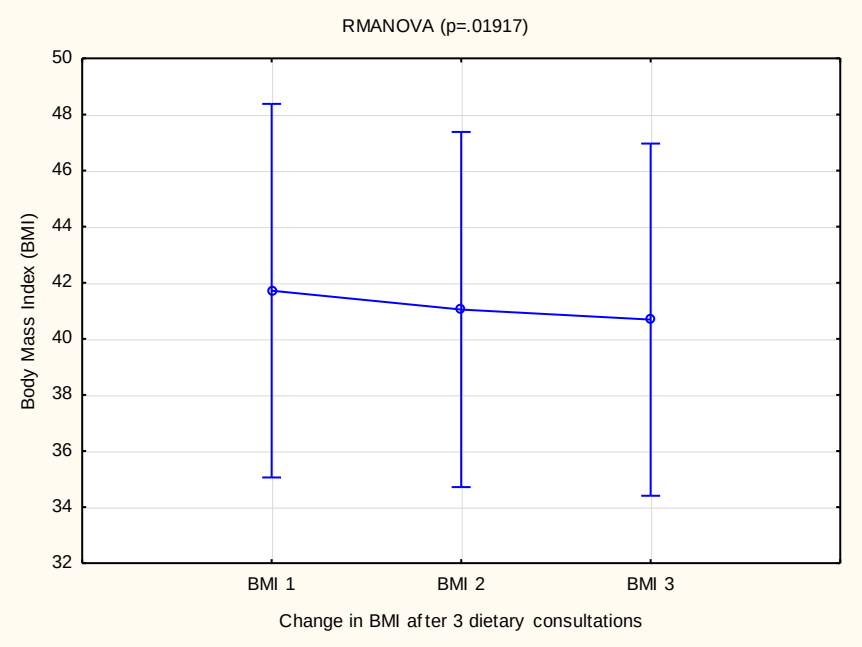

Figure 4.18: Change in BMI of study population after three dietary consultations (n=18)

There were no significant relationships between the clinical symptoms/eating behaviours and changes in anthropometrical measurements upon the second and third visits.

4.5.2 Changes in fructose intake

The 18 relevant patients showed a significant drop in fructose intake from baseline (181.5 g) to visit 2 (73.1 g). Subjects reported more than a 50% reduction in fructose intake, and, although still statistically significant, there was a slight increase in fructose intake by visit three (83.1 g).

After giving guidelines for fructose restriction, as well as comprehensive dietary counselling after history-taking (Addenda 4 and 3), the fructose intake of subjects who were seen for three dietary visits (n=18), showed statistically significant reduction (ρ = 0.001) (Figure 4.19). A Bonferroni multiple comparisons test showed that fructose 1 differed significantly from fructose 2 (p=0.0036), and fructose 1 also differed significantly from fructose 3 (p=0.0086)

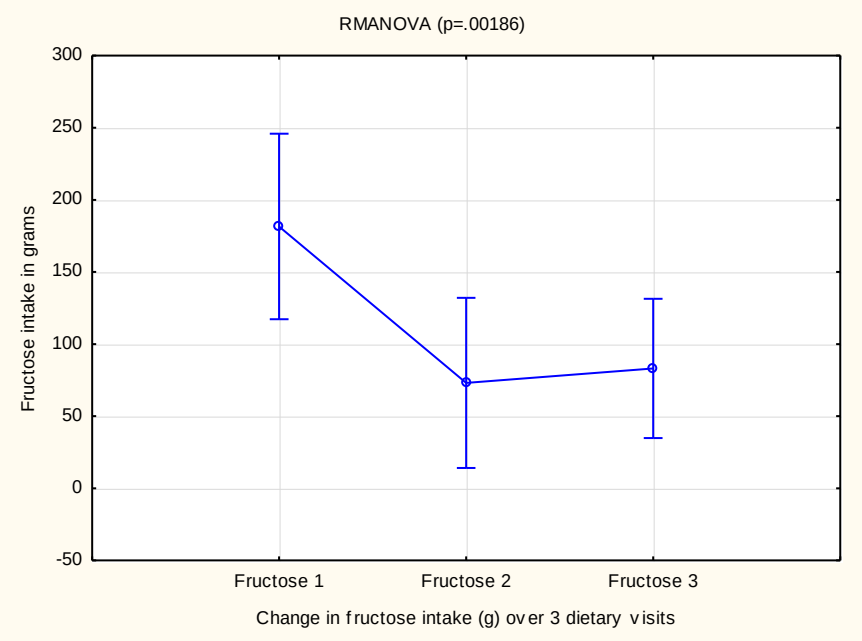

Figure 4.19: Change in fructose intake of study population over three dietary consultations (n=16)

There was no significance between fructose intake on visits 2 and 3, and reporting of any clinical symptoms/eating behaviours.

After three visits for dietary consultation, the BMI of subjects showed statistical significance, with ρ=0.019. A repeated-measures ANOVA (RMANOVA) test was done on BMI, with fructose as covariate. The analysis was done in SAS with PROC MIXED. The assumption of compound symmetry was used as the correlation structure of observations on the same

subject over the times observed. The result indicated that, in the current study population, fructose was a covariate to BMI (ρ=0.006).

4.5.3 Relationship between baseline reporting of clinical symptoms / dietary behaviours and change in BMI

Repeated-measures ANOVA tests were done to establish whether the baseline 'yes'/'no' reporting to clinical symptoms and dietary behaviours showed any relation on the change in BMI over three visits (n=18).

The baseline reporting of burning feet had no significant relation to change in BMI over three visits (ρ=0.9). No significant relationship between reporting of cravings and change in BMI over three visits (ρ= 0.8) was found (Figure 4.20).

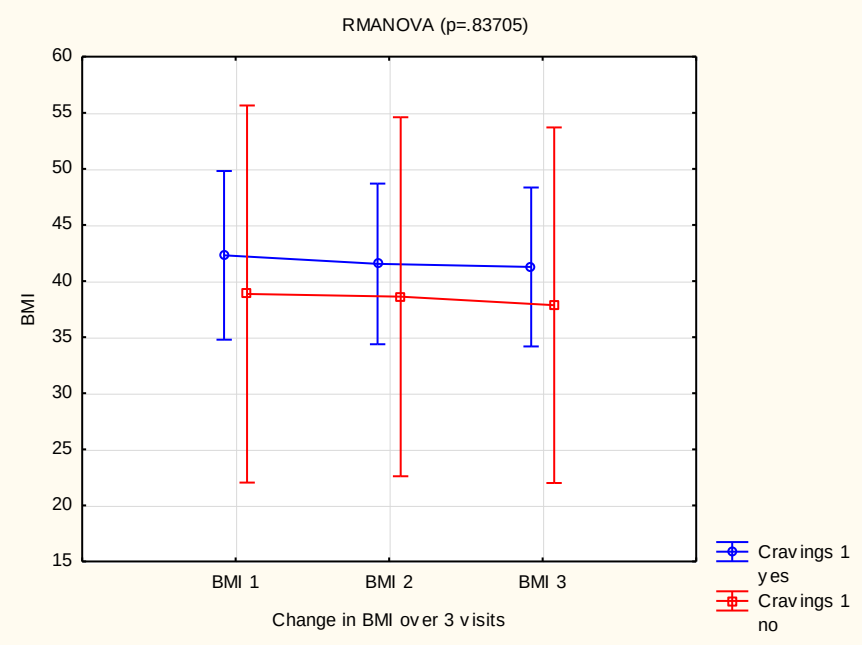

Figure 4.20 Change in BMI of study population over three dietary consultations, in relation to baseline reporting of cravings (n=18)

No relationship between fatigue and changes and BMI could be shown (ρ=0.3). No relationship was found between reporting of symptoms of irritable bowel, hypoglycaemia, frequent hunger or perception of mood swings and changes in BMI.

Figure 4.2 shows the statistical relationship (ρ=0.02) between changes in BMI over three visits and the baseline 'yes'/'no' reporting of frequent waking. The subjects who reported 'no' to frequent waking had a significant reduction in BMI over three visits, as opposed to those reporting 'yes' to frequent waking. A Bonferroni multiple comparisons test showed that in the group of subjects that reported "no" to frequent waking, BMI 1 differs significantly from BMI 2 (p=0.03) and BMI 1 differs significantly from BMI 3 (p=0.03).

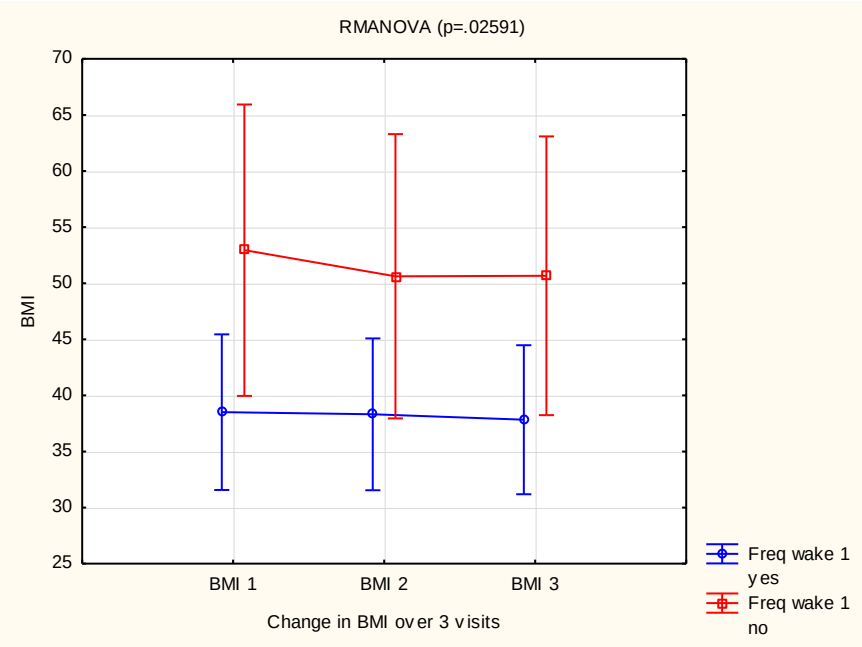

Figure 4.21: Change in BMI over three dietary consultations, in relation to baseline reporting of frequent waking (n=18)

A significant relationship was found between change in BMI over three visits and reporting of night eating. (ρ=0.002) (Figure 4.22). The subjects reporting 'no' to night eating had a significant change in BMI over three visits, as opposed to the subjects baselinely reporting

'yes' to night eating. A Bonferroni multiple comparisons test showed a significant difference between BMI 1 and BMI 2 (p=0.007) as well as between BMI 1 and BMI 3 (p=0.0008).

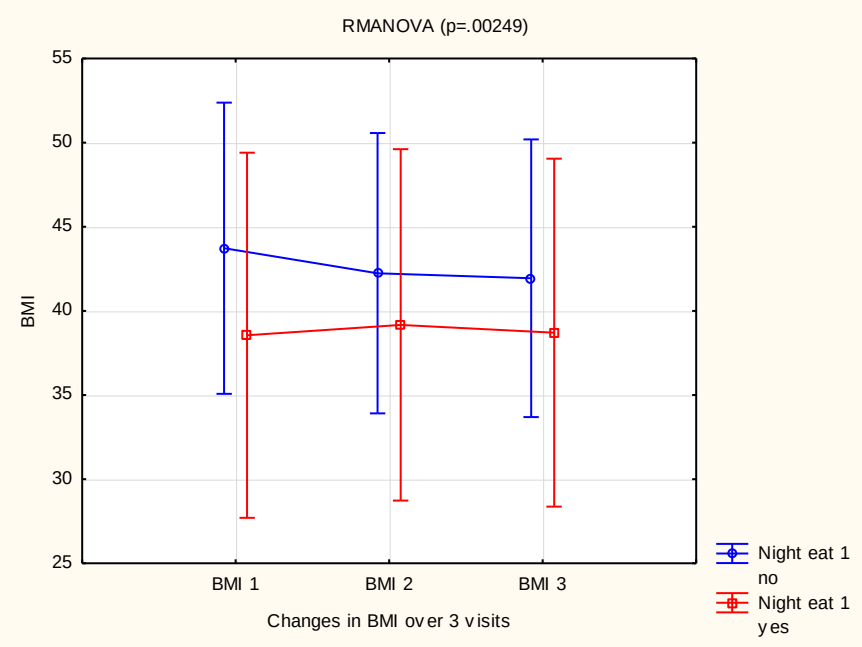

Figure 4.22: Change in BMI of study population over three consultations, in relation to baseline reporting of night eating

Dietary habits, such as daily take-away meals from fast-food outlets, unhealthy snacking and late-evening snacking were assessed, but no relationship between baseline reporting of the habits and BMI over three visits could be shown.

4.6 FERTILISATION RESULTING IN PREGNANCY

Very little data were available on the incidence of pregnancy amongst the study population, and no statistical inferences could be made as to improvement in the fertility of the study population.

CHAPTER 5

DISCUSSION

5.1 INTRODUCTION

Accurate dietary intake is well documented as being difficult to measure in a research setting with free-living subjects.[27] Underreporting is well known in almost all areas of medical history-taking, and should not be underestimated. In the particular population group studied, diet and lifestyle reportedly ranked low on the list of priorities in preparation for pregnancy, and patients wanted drug intervention instead of having to change poor dietary habits as aid in fertilisation. The patients generally also did not report a desire to improve their body weight or appearance, and the nutritional value of food was disregarded in favour of taste.

The general disregard for dietary and lifestyle improvements poses a problem to the treatment of infertility, bearing in mind that weight loss is an international recommendation as the first-line treatment for infertility.[1,2] A need exists for patients to be informed of the importance of professional dietary intervention and management, as well as to have pointed out the failure rate of self-dieting. The importance of professional guidance to enhance the fertility process and to prevent metabolic complications should also be emphasised with such patients.

The baseline aim regarding the numbers used in the study was to assess 120 subjects, in order to reach a power of 90%. However, time and financial constraints necessitated termination of the study after 18 months (November 2009 – April 2011), and the number of subjects recruited, consistent with the inclusion criteria, was 86.

A. BASELINE DATA

The baseline BMI of the study population raised concern. Although subjects with a BMI of 27 kg / m^2 and more were included in the study, all patients attending the infertility clinic for the mentioned period of time were evaluated by the researcher. Only four patients were excluded from the study due to them having a BMI of below 27 kg / m^2. Of the study population, 95% presented with BMI values considered as obese (> 30 kg / m^2),[40] and only 5% presented with BMI values between 27 and 30 kg / m^2. Effectively, the entire study population was obese, and at risk of developing health problems.[40]

The WC of the study group raised further concern, in that the trend was strongly towards having a WC wider than 90 cm. Only five patients had a WC between 80 cm and 90 cm, pointing towards the strong trend in obesity and MS of said population. Failure of the WC and WHR to show significant reduction can be explained. The waist area is difficult to measure with accuracy, especially in obese patients with excessive tissue in the midline. Although the average of three measurements was taken, the accuracy of the actual measurements remains questionable. The WHR probably showed no difference because the 'apron effect' made it difficult to measure hip circumference accurately in most patients (n=73), precluding accurate calculation of the WHR. In this regard, the methodology was poor, and the measurement inaccurate.

The presence of clinical symptoms showed some resemblance to that which is reported in the literature.[3] Hypoglycaemia was reported by less than 50% of said overweight PCOS population. The percentage is slightly more than that which was reported by Herriot et al.,[3] who found hypoglycaemia reported in 43% of obese PCOS cases (n=88), and more frequently by lean PCOS sufferers. Cravings were reported by 79% (vs. 70% of all PCOS patients, with a similar finding between lean and obese patients).[3] Symptoms of irritable

bowel were reported by 82% (vs. 68% in the literature)[3] and fatigue by 69% (vs. 82% reported in the literature)[3]. Unhealthy eating practices were reported by 85% and more of the study population. Of the study population, 99% reported a daily intake of unhealthy snacks, and 91% reported daily consumption of meals from fast-food outlets.

Whether the patients had been referred for professional dietary counselling before, or had previously chosen to try to lose weight on their own, the success rate in either case was extremely poor. Again, the finding points to the lack of information amongst overweight PCOS subjects regarding the importance of receiving professional dietary management, for the entire treatment period until fertility is reached.

The baseline average fructose intake of the study population was 167.4 g / day. The typical westernisation of diets is reported to have increased the daily fructose consumption to between 85 and 100 g / day,[12,13] and a study by Vos et al. found US adolescents between ages 12–18 years to have the highest fructose intake of 72.8 g / day.[14] The human intake of fructose that is reportedly metabolised beneficially is 16–20 g / day,[12] which roughly translates to 3 servings of whole, fresh fruit per day. The baseline mean fructose intake of subjects in the study was, therefore, almost twice as high as were the estimations in the previous literature. The amount of fructose concerned would tend to play a major role in the development of reduced insulin sensitivity and IR.[12]

No significant relationships were found between BMI and baseline symptoms or eating behaviours. Although a seemingly large difference existed between the BMI of subjects answering to daily unhealthy snacking, only one patient answered ‘no’ and the BMI of the patient concerned was 10 kg / m^2 higher than was that of the rest of the population who answered ‘yes’.

WC showed stronger relationships with reporting of baseline symptoms and eating behaviour. The larger the WC, the stronger the relationship with snoring (ρ=0.02). Baseline reporting of daily food intake from fast-food outlets positively related with larger WC, although not significantly (ρ=0.06). Of the subjects (n=74), 91% reported daily intake of fast foods.

No statistically significant relationships were found between WHR and any of the mentioned clinical symptoms / eating behaviours, except for the reporting of urgent hunger, in relation to which a higher WHR related positively to more 'yes' answers. Because of the poor methodology used for WHR, no comment on the finding could be substantiated.

Statistical significance was shown, by means of the use of the Spearman rank-order correlation test, between baseline intake of fructose and weight (ρ=0.0373). The correlation was significant (ρ=0.0373), although weakly negative (r=0.225), and the variation in weight contributed by fructose accounted for 5.06%. The reader has to bear in mind that, baselinely, 95% of patients were obese, and the mean baseline fructose intakes of the subjects were extremely high. The fructose intake was only calculated in relation to edible fruit and sweetened soft drinks and fruit juices, as reported by subjects, and would probably have been higher, had other sources of added fructose been taken into account. Given the high intake of unhealthy snacks and meals from fast-food outlets, said finding is surprising, as WC showed a near-significant relation to reporting of daily fast-food intake. No scientific explanation can be given for the negative correlation found between weight and fructose intake.

Contrary to the negative correlation between baseline fructose intake and weight, there was a strong positive relation between 'yes' reporting to burning feet and fructose intake (ρ=0.02). This is not substantiated by literature.

No relation existed between baseline fructose intake and reporting of cravings (ρ=0.86). Derangements in appetite control hormones were known to exist in patients with PCOS.

Moran et al.[16] had previously shown that postprandial satiety is lower and postprandial hunger is higher, before and after weight loss, than in weight-matched controls. The literature provides an abundance of evidence that disturbances in appetite regulation in PCOS probably accounts for the reported disturbances in hunger and satiety signals.[4,5,6,15,16]

The subjects reporting 'yes' to night eating tended towards a positive relation to baseline fructose intake (ρ=0.07). A strong positive relationship was found to exist between baseline reporting of frequent waking and baseline fructose intake (ρ=0.02), and a highly significant relationship was found between daily intake of food from fast-food outlets and baseline fructose intake (ρ=0.01).

Rationally, the 'night eaters' and the 'frequent wakers' might be the same set of subjects, and reports of drinking substantial amounts of sweetened beverages and juices during the course of the night were frequent, although the timing of the beverage consumption was not recorded precisely. The strong positive relationship between fructose intake and the consumption of meals daily from fast-food outlets has already been addressed during the discussion on WC and the daily intake of fast foods. No previous research in scientific literature could be found to compare, or substantiate, the findings of the current study.

B. FOLLOW-UP VISITS

The dropout rate of 50% after the first visit, with a further 41% after the second visit, exceeds the dropout rates of 26–38% documented by Moran et al.[6] The baseline study population included 86 patients, the first follow-up visit (visit 2) included 43 patients, and by the second follow-up visit (visit 3) 18 patients were left for assessment. The patients baselinely recruited were briefly informed by the attending infertility specialist that they had to see a dietician, which information was generally unexpected by the patient. A discussion of the primary

importance of improvements to diet and lifestyle as a first-line therapy was not emphasised or adequately explained before the dietary consultation, which could further contribute to the dropout rate. The medical staff involved in treatment of overweight PCOS sufferers encouraged weight loss, but the importance of weight management by a professional dietician experienced in PCOS lacked due emphasis.

Significant reduction in the weight of patients was only shown after 3 visits (n=18) (ρ=0.017). Although a weight loss of 4 kg was shown upon the second visit (n=43), the loss concerned was not significant (ρ=0.09). Upon the third visit (n=18), the total loss of weight was 2.58 kg, but, for the given population size, the finding was statistically significant (ρ=0.017). The reader should bear the difference between statistical significance and practical significance in mind. The loss of weight required for improved fertility treatment and reduced health risk is estimated at 5–10% of baseline weight, although the BMI of the subject might still be in the obesity range.[2,5,15] The loss of 2.58 kg after three visits for dietary management constituted a 5% loss of baseline weight in a 50-kg woman, while the lowest recorded weight in the specified study population was 60 kg. Although weight loss reached statistical significance after three visits, the actual amount of weight lost was far less than had been expected. The same applies to the statistically significant reduction in BMI (ρ=0.019) seen over three visits (n=18). The overall reduction of BMI was a mere 1.1 kg/m^2, but was statistically significant in the sample size.

After three visits for dietary management (n=18), there was a highly significant reduction in the amount of fructose intake of almost 100 g / day (p=0.001). The goal daily intake of

20 g[12] was still being exceeded, showing a daily fructose intake of 78 g. Since methodology for calculation of total fructose intake was unavailable, a calculable intake of 20 g fructose per day would still mean that the total daily intake is higher than it should be.

The reduction was both statistically and practically significant, corresponding better to estimated values for high fructose intake, as cited in the scientific literature.[12,14] A repeated-measures ANOVA test was done on BMI, with fructose as covariate. In relation to the current study population, it can be said with certainty (ρ=0.006) that fructose was a covariate for BMI. The finding raises a further question regarding the finding that baseline fructose intake and baseline weight are negatively correlated. Weight is a necessary variable for calculation of BMI, and the higher the weight, the higher the BMI. If fructose played a covariate role in the BMI of the subjects in the current study, the correlation between baseline fructose and weight should have been positive.

The statistical significance shown between BMI changes and baseline answering for 'frequent waking' and 'night eating' poses an interesting dilemma. Patients who answered 'yes' to both frequent waking and night eating on baseline assessment showed no significant change in BMI, whilst the patients that answered 'no' to both the behaviours went on to reach a significant reduction in BMI. As mentioned, it stands to reason that the 'frequent wakers' are likely to be the 'night eaters'. Affirmation of the two behaviours might be viewed as predictive of future failure to reduce BMI significantly.

One might ask whether the frequent waking and night eating might not be related to the presence of sleep disturbances. The results of the current study have already shown that a larger WC relates positively to larger incidence of snoring. Having shown that both nightly eating and frequent waking pose a threat to reducing the BMI, the two behaviours could be linked to the WC of the patient, with the inference of a possible positive relation between sleep disturbance and all factors mentioned that relate positively to WC and BMI.

Although the intake of fructose was significantly reduced at visit 3, the goal daily intake of 20 g[12] was still being exceeded, showing a daily fructose intake of 78 g. Since methodology

for calculation of total fructose intake was unavailable, a calculable intake of 20 g fructose per day would still mean that the total daily intake is higher than it should be. Lists of fructose contents of foods and beverages are, as yet, incomplete, and the South African Food Composition Tables do not list the fructose content of any foods.

The strong elements in the results of the current study are based on the tendency of the particular population under survey to avoid dietary restriction, shown by the high dropout rate.

The results of the current study show that many factors have the potential to hinder weight loss in overweight PCOS sufferers seeking fertility. The journey of each patient is multifaceted, and much understanding of all aspects is needed. The dietary management of each patient should be regarded as a 'coaching' process, which requires on-going follow-up, until such time as the desired outcome (in the present case, fertility) is reached.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The optimal dietary treatment to bring about permanent weight loss for treatment of overweight/obese PCOS sufferers has long been an elusive one, causing much frustration on the part of both patient and infertility specialist. No doubt should remain in the mind of the reader that weight loss offers the most effective answer to the particular fertility problem concerned. Unfortunately, self-knowledge about weight loss has been super-commercialised, and, in the specialised field of nutrition and weight loss, patients are encouraged by various media and commercial diets that they can lose weight on their own. In the current study, as well as in related others,[6,24] it is clearly shown that such is not, however, the case. The odds ratio for dietary and lifestyle intervention clearly outweighs that for single or combined drugs, as well as that for artificial reproductive therapy.[2]

The value of detailed dietary history-taking cannot be underestimated in overweight patients with PCOS. Such forms the basis of the trust relationship between dietician and patient, and enables the dietician to coach the patient on a regular basis, in order to facilitate faster weight loss and fertilisation outcome than might otherwise be gained.

The first hypothesis of the current study, stating that chronic fructose consumption is not associated with the anthropometric and clinical markers in obese women with PCOS, is rejected on the following bases:

- Chronic fructose consumption, as assessed at baseline, was shown to affect the BMI and WC, as well as clinical symptoms, such as burning feet.

- Behaviours that seemed to be influenced by excessive fructose consumption were frequent waking and night eating.
- The significance of excessive fructose consumption on symptoms and eating behaviours disappeared after the first visit for dietary management, during which restriction of the intake of fructose was advised.

The second hypothesis, stating that restriction of fructose will not influence the adherence to a diet designed to bring about weight loss, could not entirely be rejected. No certainty was obtained on whether the high dropout rate was due to restriction of fructose intake, as the patients who adhered to the dietary guidelines showed both a reduction in fructose and in BMI.

6.2 RECOMMENDATIONS

The recommendations of the current study were as follows:

1. The need for at least three visits to a dietician being required in the treatment of overweight PCOS is the primary recommendation. The dietician concerned should form part of the immediate team managing overweight/obese PCOS patients, and should keep channels of communication open with all specialists and staff involved.

2. Furthermore, the patient should be made to realise that, if the dietary treatment, as suggested, is not followed or complied with by at least showing clinically significant weight loss on a three-monthly basis, further fertility treatment will be delayed, until such weight loss occurs.

3. From the literature overview,12 and also with fructose having been shown as a definite covariate to BMI in the current study population, striving for a measurable

fructose intake of 20 g per day, preferably from edible fruit, should be a dietary goal, for purposes of improved weight loss.

4. Patients should be questioned about their sleeping habits and nightly eating. Nightly eating should be strongly discouraged, and the patient should be reassured that, with improvements in diet and lifestyle, and with reduction in BMI, their sleeping pattern will improve. Taking such a step might also prove to be an incentive for the patient to want to improve their lifestyle and to lose weight.

5. General educational tools to enable patients and fellow colleagues to manage fructose overload should be developed. It is suggested that the broad guidelines should include:

- caloric reduction to bring about a loss of at least 500–750 g / week;
- reduction of both the GI and GL of the diet in general, by means of:
  - advising the patient to omit starch from the evening meal, replacing it with vegetables, which should serve as a practical guideline for reducing the GL of the diet;
  - recommending the use of alternative sources of starch, with a lower GI, to replace existing high GI starches;
  - seriously discouraging unhealthy food intake and the consumption of take-away meals, in order to reduce caloric and trans- and saturated fat intake.[4,21]
- the use of inexpensive, but lean, protein of high biological value in meals in order to minimise postprandial insulin excursions, and so as to enhance satiety.5,15
- omitting sweetened liquid beverages of any kind, with particular emphasis on avoiding fruit juices, allowing only the consumption of water, sparkling water, diet drinks and unsweetened tea/coffee;

- omitting all sources of dried fruit; and
- reducing fructose intake to no more than 3 servings of fresh pieces of edible fruit, so as not to exceed a measurable fructose intake of 20 g / day. The fruits may be spaced throughout the day as the patient desires.

6. Strict dietary regimes should be avoided, as the patient has to learn to adopt improved eating habits as a lifestyle.

7. The patients should also be encouraged to bring their partner/spouse to sit in on the full first consultation, as the couple should improve their lifestyle together, and PCOS sufferers require support in their endeavour to lose weight and to improve their lifestyle.

8. Compilation of a comprehensive list of the fructose contents of fruit, commonly consumed processed foodstuffs and sweetened beverages should be undertaken in South Africa, in order to aid in the accurate fructose calculation of the individual diets of all patients.

9. The overweight patient suffering from PCOS who seeks fertility has to take ownership of their dietary predicament that has led to the associated high health risk and to the hindering of fertility. Professional dietary management should be insisted upon by infertility specialists, as soon as the diagnosis is made of PCOS, especially for those who are overweight/obese. Strictly overseeing that the patient is actually seeking professional dietary management should form part of the medical management of patients in said category.

CHAPTER 7

SHORTCOMINGS OF THE STUDY

The following are the shortcomings of the current study:

1. The envisaged population size for a high-powered study of 90% (ρ=120) was not reached within the set study period.

2. The population group was generally ill-informed about the importance of dietary changes to facilitate weight loss, and showed apathy regarding the nutritional value of their daily dietary intake.

3. Very few partners/spouses attended dietary consultations, although doing so was encouraged by the researcher upon the first visit of the patient concerned.

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ADDENDA

ADDENDUM 1: ANTHROPOMETRIC AND BIOCHEMICAL PROGRESS SHEET

	ADDENDUM 1: ANTHROPOMETRIC AND BIOCHEMICAL PROGRESS SHEET					
PATIENT NAME:					FILE NO.:	
	ADDENDUM 1 : PROGRESS SHEET					
Diagnosis	Scan	YES	NO		Patient name:	
	Anovulation	YES	NO			
	Androgen levels	YES	NO		File no.:	
		0/12	3/12	6/12	9/12	12/12
Examination	Height					
	Weight					
	BMI					
	WC					
	Hips					
	WHR					
Fasting bloods	Insulin					
	Glucose					
	HDL					
	LDL					
	Triglycerides					
	Total cholesterol					
	CRP					
	TSH					
	Prolactin					
	FSH					
	Testosterone					
	SHBG					
	Free testosterone					
	17-OH-Progesterone					
	Cortisol					
	DHEA-S					

ADDENDUM 2: SUBJECTIVE CLINICAL SYMPTOMS

A. SUBJECTIVE CLINICAL SYMPTOMS

Date: _____ File no: _____

Name _____ Age: _____

Please answer the following questions by circling the option that best suits you.

1. Do you ever experience 'dizziness' or 'shakiness', which goes together with a need for something to eat? YES NO

2. Do you experience cravings for certain foods? YES NO

3. Name a few of the foods for which you crave (if the answer to the previous question was YES): _____

4. Do you frequently become hungry? YES NO

5. Is your hunger painful and urgent? YES NO

6. Do you often become irritable or moody? YES NO

7. Do your feet burn? YES NO

8. Do you sometimes get up at night to eat? YES NO

9. Do you experience bloating/flatulence? YES NO

10. Are you prone to constipation? YES NO

11. Do you experience frequent headaches? YES NO

12. Are you very tired during the day? YES NO

13. Do you snore when you sleep? YES NO

14. Do you wake up frequently during the night? YES NO

15. Are there certain foods that upset your stomach? YES NO

    Name some of these foods: _____

B. SUBJEKTIEWE KLINIESE SIMPTOME

Datum: _____ Leêr no: _____

Naam _____ Ouderdom: _____

Beantwoord asb. die volgende vrae deur die beste antwoord te omkring.

1. Ervaar u ooit bewerigheid wat gepaardgaan met ’n sterk behoefte om iets te eet? JA NEE

2. Ervaar u ooit ‘cravings’ na sekere voedsels? JA NEE

3. Noem ’n paar van hierdie voedsels (indien u antwoord JA was vir die vorige vraag):
_____

4. Word u baie gereeld honger? JA NEE

5. Is u honger dringend en pynlik? JA NEE

6. Word u gereeld buierig? JA NEE

7. Brand u voete soms onder u voetsole? JA NEE

8. Staan u soms snags op om iets te gaan eet? JA NEE

9. Voel u baie keer winderig en opgeblase? JA NEE

10. Word u maklik hardlywig? JA NEE

11. Ervaar u gereelde hoofpyne? JA NEE

12. Is u baie moeg gedudurende die dag? JA NEE

13. Snork u wanneer u slaap? JA NEE

14. Word u gereeld wakker deur die loop van die nag? JA NEE

15. Is daar sekere voedselsoorte wat u maag ontstel? JA NEE

Noem asb. ’n paar voorbeelde: _____

ADDENDUM 3: PATIENT INFORMATION AND HISTORY

Patient name:	Date:
Age:	Referring doctor:
Weight history:	Family history:
Previous dietetics referral:	Success:
Previous self-dieting:	Success:
Medication:	Supplements:
Bowel movements:	
Skin tags:	Acanthosis nigricans:

NOTES:

DIET HISTORY:	WEEKENDS
Breakfast:	
Morning snack	
Lunch:	
Afternoon snack:	
Supper:	
Late snack	

:

FOOD FREQUENCY TABLE

FOOD:	***FREQUENCY***
Red meat	
Chicken	
Fish	
Milk / Condensed milk / Coffee creamers	
Yoghurt/Ice-cream	
Eggs	
Cheese	
Bread	
Fruit	
Vegetables	
Condiments	
Chips/Nuts/Snacks	
Cool drinks / Fruit juice	
Tinned foods	
Fats	
Sugar/Sweeteners/Honey	
Biltong / Dried sausage	
Biscuits	
Sweets/Chocolates	
Alcohol	
Eating out / Take-aways	

ADDENDUM 4: DIETARY GUIDELINES TO PATIENTS DURING ONE–ON–ONE CONSULTATION

The dietary guidelines for the current research study were designed to provide the patient with a daily intake of roughly 1 400 kcal, consisting of approximately 30% protein, 40% carbohydrate, and 30% fat.[15] As first line in lifestyle improvement, and after acquiring insight into her eating habits and lifestyle, through the taking of a detailed history, as per Addendum 1, the eating pattern and macronutrient content of the patient's diet were addressed, rather than the promotion of quick weight loss.[5] The guidelines were designed to restrict fructose intake to

16–20 g per day,[12] but will serve to restrict total sugar intake, facilitating calorie restriction in general, and enhancing the patient's protein intake to improve glucose tolerance, to reduce postprandial insulin excursions, and to facilitate satiety.[26] Emphasis will also be placed on improving the quality of insulin-mediated glucose intake in the form of starch (GI), and on advising the lowering of the glycaemic load (GL) of starches.[3,4,15]

1. The consumption of no soft beverage or fruit juice whatsoever (not even diluted) is allowed, including:

   - sweetened fizzy or gassy cool drinks;
   - flavoured water, either sparkling or still;
   - sports drinks of any kind;
   - energy drinks of any kind;
   - any form of fruit juice (even though it might be labelled 'pure' or 'sugar- free'); and
   - any form of concentrate that is mixed with water.

   The alternative to the above drinks is ordinary tap water, still or sparkling bottled water without any flavourants, or the occasional 'diet' drink, should the patient so desire.

2. No form of dried fruit was allowed to be consumed for the duration of the study, for the purpose of fructose restriction. Two to three servings of fresh, whole fruit is the

only form of fructose allowed (constituting 16–20 g fructose) daily,12 and only single fruit servings, spaced at least 4 hours apart, were allowed. The servings may have been eaten as a snack between meals, or as an accompaniment to meals. The portion size of a single serving of fruit was explained to the patient according to the exchange lists for meal planning.

3. Consumption of starch-based products with a high GI was discouraged, and patients were provided with a guideline to help them orientate products according to the GI. The patients received advice on dividing their daily allowance for low GI starch between breakfast, lunch and supper.

4. The inclusion of at least 1–2 portions of lean animal protein with each meal was emphasised, especially with regards to improving satiety of meals, and attenuating the glucose and insulin excursions after meals.25

5. The patients were encouraged to eliminate their intake of daily calories through snacks and drinks.5 Illustrations of more substantial meals were given and discussed (bearing in mind the patient's particular daily work set-up, etc.), in order for meals to last longer and so as to discourage snacking.

6. The importance of curbing saturated fat and trans fat intake was explained to the patient,21 and the sources of the fats discussed. Practical guidelines were given on how to minimise intake, and healthier alternatives discussed.

7. All suggestions and advice were accompanied by using example meals for illustration, and, throughout, the researcher referred back to the patient's own dietary history/recall, in order to emphasise where changes had to be made.

8. The daily intake of the patients will be roughly based on the following:

Food type	No. of portions	No. of kcal
Protein (medium-fat)	4	375
Starches	4	320
Fruit	2	120
Vegetables	3–4	80
Fats	4	180
Milk (low-fat)	2	240

9. Weight loss/maintenance, and changes in waist circumference and WHR, indicated adherence to the guidelines within the first month, as well as will subjective feedback from the patient on subjective symptoms.

10. The patient was instructed on including moderate exercise, starting with 30-minute sessions of brisk walking or cycling, 3–4 times per week. With each follow-up visit, the patient received instructions to increase the duration of the chosen activity, increasing the frequency to at least 5 times per week.5

ADDENDUM 5: DIETARY FOLLOW-UP

Fructose intake: _____ ***Date:*** _____

	Amount per day
Soft beverages / sports drinks / juices:	
Alternatives:	
Dried fruit:	
Fresh fruit:	
Spacing of fruit:	
Total amount of table sugar per day:	
Improvements in GI	
Which foods have improved regarding GI?	
Composition of supper	
Starch content of supper:	
Inclusion of protein with meals:	
Snacking	
Frequency:	
Kinds of foods used as snacks:	
Late-evening snacking:	
Saturated/trans fats (Take-Aways):	

DIET HISTORY:	WEEKENDS
Breakfast:	
Morning snack	
Lunch:	
Afternoon snack:	
Supper:	
Late snack	

ADDENDUM 6: PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

Title of the research project:

The role of fructose restriction in addition to dietary modifications for weight loss and lifestyle improvement, on fertility outcome and other markers of metabolic syndrome, in obese women with PCOS

Principle investigator: Annchen Weidemann
Consultant Dietician (RD (SA))

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Pinelands
7405

Contact numbers: 021 5320640 / 083 3043257

INFORMATION FOR THE PARTICIPANT

You have been invited to take part in a research study. Please take some time to read the information presented here, which will explain the details of this project. Please do not hesitate to ask the dietician any questions that you have about any part of the study that you do not understand. It is of much importance that you are fully satisfied and that you clearly understand what this research entails, and how you could be involved therein.

Your participation is entirely voluntary, and you are free to decline to participate in the study. If this is your decision, you will not be negatively affected in any way whatsoever. You are also free to withdraw from the study at any point, even if you baselinely agree to take part in it.

This study has been approved by the **Committee for Human Research at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, the South African Guidelines for Good Clinical Practice, as well as the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this study all about?

As the title states, this study is firstly about helping women who suffer from polycystic ovarian syndrome (PCOS), and who are classified, according to the BMI, as obese. It is a well-known fact that weight loss is the cornerstone to treating this condition, and many studies have been done, using conventional 'dieting' to try to achieve weight loss.

This study is mainly about using a more comprehensive, guideline-oriented approach to weight loss, with the added feature of fructose restriction, which has received much attention in recent literature, for its ability to cause much the same metabolic picture as does PCOS. The main aim of the study is to evaluate whether minimising fructose intake helps with weight loss, and whether such minimisation might speed up the improvement of metabolic features that is typical of your syndrome.

The study was decided upon, after the above-mentioned particular approach seemingly delivered noticeable improvements in obese PCOS patients, both regarding fertility and weight loss. No claims or statements can be made of a superior approach if a sound, scientific study has not been executed to test this notion.

Why have you been invited to participate in the study?

You have been diagnosed with PCOS, and present with a BMI of more than 27, which makes you an ideal candidate for this particular study. Your response to the dietary treatment you are about to receive will help to provide the researcher (Annchen Weidemann), as well as your infertility specialist, with valuable information, in order to improve our treatment of this particular infertility syndrome. It will also enable us to help other women like yourself better than might otherwise be possible.

What will your responsibilities be?

All procedures and processes that you will experience during the study are routinely done when you attend the Infertility Clinic at Tygerberg Hospital.

In relation to the study, all that you need to do is to adhere as best you can to the comprehensive advice and guidelines you will be given by the presiding dietician, and adhere to follow-up visits with her, on the same day as you are routinely scheduled for follow-up with your infertility specialist.

You will be followed up on routinely every three months by your infertility specialist.

Are there any <u>additional</u> costs involved?

No, there will be no additional costs involved beyond what you would normally have paid as an outpatient at Tygerberg Hospital.

Are there risks involved in taking part in this research?

The dietary guidelines with which you will be provided are designed from in-depth research, and will not cause you harm in any way.

If you do not agree to take part in the study, what alternatives do you have?

You may choose to attend a dietician of your own choice, or you may choose to follow an alternative diet of your choice, in order to achieve the weight loss that is necessary to facilitate your fertility. You may request to be referred to one of the dieticians at the Department of Human Nutrition, Stellenbosch University.

Who will have access to your medical records?

The information collected will be treated as confidential and will be protected. Should the data be used in a thesis or a publication, your identity will remain anonymous. Only the researcher (the dietician, Annchen Weidemann), and your infertility specialist, as well as nursing staff who have been trained to treat all related information as confidential, will have access to your personal information.

Is there anything else that you should know or do?

If you should so desire, you may inform your family practitioner that you are taking part in a research study. You may contact the Committee for Human Research at 021 938 9207 if you have any concerns or complaints that have not been adequately addressed by your study researcher.

You will receive a copy of this information and consent form for your own records.

Interpreter

The staff and researcher (consultant dietician, Annchen Weidemann) at the Infertility Clinic at Tygerberg Hospital are fully bilingual with regards to Afrikaans and English, and an interpreter for isiXhosa will be made available upon request, although the researcher does have a reasonable command of the isiXhosa language, after having lived and worked in the Eastern Cape and having received isiXhosa training.

Declaration by participant

By signing below, I …… agree to take part in a research study entitled:

The role of fructose restriction in addition to dietary modifications for weight loss and lifestyle improvement, on fertility outcome and other markers of metabolic syndrome, in obese women with polycystic ovarian syndrome (PCOS).

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 about the study and all my questions have been adequately answered.
- I understand that taking part in the study is voluntary and I have not been pressurised to take part therein.
- I may choose to leave the study at any time, and shall neither be penalised nor prejudiced in any way.
- I may be asked to leave the study before it has finished, if the researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (place) ……. on (date)…… 2009

……. ……

Signature of participant **Signature of witness**

Declaration by investigator

I (name) …… declare that:

- I explained the information in this document to …….
- I encouraged her to ask questions and took an adequate amount of time to answer them.
- I am satisfied that she adequately understands all aspects of the research, as discussed above.
- I did / did not use an interpreter. *(If an interpreter is used, then the interpreter must sign the declaration below).*

Signed at (place) …… on ……. (date).

…….. ……

Signature of investigator **Signature of witness**

Declaration by interpreter

I (name) …….. declare that:

- I assisted the investigator (name) ……. to explain the information in this document to (name of participant) ……., using the language medium of Afrikaans/isiXhosa.
- We encouraged her to ask questions and took an adequate amount of time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all her questions satisfactorily answered.

Signed at (place) …… on ……. (date).

…….. ……

Signature of interpreter **Signature of witness**

ADDENDUM 7: PERSONAL OBSERVATIONS

- Although specific foods, other than fructose-containing drinks and fruit, were not monitored for the purposes of the current study, the tendency of the study population to eat unhealthy meals and snacks, based on starch and sugar, was strong. The subjects, who were ignorant of the concepts of GI27 and GL,3 and those who tried to lose weight on their own, did so by using slimming tablets and mixtures, and not by manipulating or improving their daily dietary intake.
- Although specific foods, other than fructose-containing drinks and fruit, were not monitored for the purposes of the current study, the tendency of the study population to eat unhealthy meals and snacks, based on starch and sugar, was strong. The subjects, who were ignorant of the concepts of GI27 and GL,3 and those who tried to lose weight on their own, did so by using slimming tablets and mixtures, and not by manipulating or improving their daily dietary intake.
- Although not substantiated in the scientific literature, burning feet is a symptom that the researcher had often encountered in PCOS sufferers, which was the reason for it being included in the study.
- Such eating behaviour could account for both a high intake of high GI, starch-based foods, and of such deep-fried foods as potato chips, contributing to a high intake of trans fat.11 High trans fat intake has been linked to ovulatory infertility, and has been reported as being inversely related with health consciousness.21 Meals from fast-food outlets are normally consumed together with a sweetened drink of choice, contributing to fructose intake.

- The general disregard of improvements in diet and lifestyle of the patient population of the study could partly explain the high dropout rate.
- The unwillingness of the patient population to apply dietary guidelines for weight loss became apparent in their extreme dropout rate after either referral to a dietician or when attempting weight loss alone. Attempts at previous own weight loss were typically through use of tablets or herbs, and not dietary manipulation.
- Patients frequently commented that they struggled with weight loss because of dieting being 'expensive'. The financial expense of their daily intake, as reported by the subjects, most likely would exceed the cost of improving their diet, which essentially would have required reduction in intake. The unsubstantiated claim/belief of the high financial cost of 'dieting' probably contributed to the dropout rate of the subjects concerned.
- Spouses or partners rarely attended the dietary consultations, which showed lack of support in the patients' domestic environment.
- Reports of dietary intake showed ignorance/apathy towards nutritive value of food, and fast or processed food was favoured over wholesome, cooked meals. Emphasis on the intake of sweetened beverages was characteristic of the population.
- The behaviours and beliefs of each patient, regarding nutrition and food, play an integral part in the success or dropout rate concerned.
- Patients in the study population were parents-to-be, and dietary lifestyle would impact negatively on a child born into an already insulin-resistant gene-pool. Very few of the subjects were accompanied by their partners/spouses to the interviews. The dietary habits of the partners play a significant role in the dietary habits of overweight woman with PCOS seeking fertility, and it would only be fair to insist that such partner

should accompany the PCOS sufferer on each dietary management visit, in order that they might also come to understand the same principles, in order that they might provide support.

- Although not drawn from substantial data, the subjects showed a negative attitude towards nutritive value of dietary intake, and physical appearance, with respect to obesity and body appearance, held little value. They showed preference for drug treatment to assure them of fertility, and did not expect to be exposed to dietary consultation for lifestyle improvement. The number of subjects who included their spouse/partner in the consultations was not recorded, but, according to the perception of the researcher, such inclusion was rare.