Association between the metabolic syndrome and cancer risk: The potential role of fatty acids on body composition

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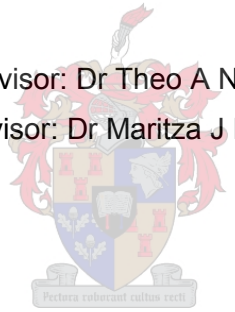

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DECLARATION

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ABSTRACT

Background: Sub-Sahara Africa is experiencing an epidemiological transition with an increasing burden of non-communicable diseases (NCDs) including obesity, hypertension, insulin resistance (IR) and dyslipidaemia. These NCD are collectively labelled the metabolic syndrome (MetS). The MetS is characterised by dyslipidaemia, and in particular, distorted fatty acid (FA) metabolism. Additionally, the MetS and its components are also associated with different FA classes. Furthermore, several lifestyle-cancers have also been associated with the MetS and its components.

Aim: To determine the association and interaction between the MetS and cancer risk and the likely influence of FAs on body composition in a female population residing in the Western Cape, South Africa.

Methods: Female farm workers in the Cape Winelands region (n=80) aged 20-60 years were randomly selected and categorised as having the MetS (n=34 MetS and n=46 non-MetS) using the International Diabetes Federation (IDF) criteria. All participants were additionally classified according to their body mass index (BMI). Blood pressure was measured, followed by blood sampling to determine blood glucose and insulin levels, as well as a full lipid profile. Selected red blood cell (RBC) membrane FAs and FA ratios were analysed, and enzyme-linked immunosorbent assays (ELISAs) were used to quantify serum insulin-like growth factor-1 (IGF-1) and leptin concentrations. Anthropometric measurements and bioelectric impedance analyses (BIA) were also performed.

Results: The prevalence of the MetS was 42.5 % with abdominal obesity (100.0 % for the MetS, and 39.1 % for the non-MetS), hypertension (82.4 % for the MetS, and 47.8 % for the non-MetS), and low high-density lipoprotein cholesterol (HDL-c) (76.5 % for the MetS, and 34.8 % for the non-MetS) being the most prevalent MetS risk factors. Several statistically significant differences were observed between the MetS and non-MetS groups for blood parameters, including insulin and HDL-c levels ($p<0.001$), and glucose, IGF-1, and leptin levels ($p<0.05$). The MetS group also presented with significantly higher anthropometric measurements, including BMI ($p<0.05$), waist circumference (WC), waist-to-hip ratio (W:H), and the sagittal abdominal diameter (SAD) (all $p<0.001$). Furthermore, BIA (including visceral adipose tissue (VAT) area, percentage VAT (VAT %) and -subcutaneous adipose tissue (SAT %), and VAT to SAT ratio (VAT:SAT) ($p<0.001$ for all) also differed between the MetS and non-MetS groups. No significant differences were noted for any of the individual FAs or FA ratios. Categorisation according to metabolic status and BMI was shown to influence several metabolic-associated blood parameters, anthropometric measurements, BIA. However, metabolic status and BMI did not influence individual FA levels or FA ratios. The obese MetS group presented with significantly higher IGF-1 levels compared to their normal weight non-MetS counterparts. Correlation analyses indicated several significant associations between anthropometric measurements, BIA, FAs and metabolic-associated blood parameters.

Conclusion: The results from this study suggest that metabolic status alone, and the combined effect of metabolic status and BMI, may predict alterations in metabolic-associated blood parameters, anthropometric measurements, and BIA in women, possibly linking obesity and the MetS to an increased risk of developing lifestyle-associated cancer.

Keywords
Metabolic syndrome, fatty acid profile, body composition, leptin, cancer risk

OPSOMMING

Agtergrond: Tans ondervind Sub-Sahara Afrika ‘n epidemiologiese oorgang met toenemende las van nie-oordraagbare siektes (NOS’s) insluitend vetsug, hipertensie, insulienweerstandigheid (IW) en dislipidemie. Hierdie NOS word gesamelik na die metaboliese sindroom (MetS) verwys. Die MetS word gekenmerk deur dislipidemie, en veral ‘n verwronge vetsuur (VS)-metabolisme. Addisioneel word die MetS en komponente daarvan ook met die verskillende VS klasse geassosieer. Verskeie leefstylkankers word voorts ook met die MetS sy en komponente geassosieer.

Doel: Om die verband en interaksie tussen die MetS en risiko vir kankerontwikkeling te bepaal, en die moontlike invloed van VSe op liggaamsamestelling in ‘n vroulike populasie wat woonagtig is in die Wes-Kaap, Suid-Afrika.

Metodes: Vroulike plaaswerkers van die Kaapwynland distrik (n=80) tussen die ouderdomme 20-60 jaar, is ewekansig gekies en geklassifiseer met die MetS (n=34 MetS, en n=46 non-MetS) deur van die Internasionale Diabetes Federasie (IDF) kriteria gebruik te maak. Alle deelnemers is addisioneel volgens hulle liggaamsmassa indeks (LMI) geklassifiseer. Bloeddruk is bepaal, gevolg deur ‘n bloedmonster om bloedglukose, insulienkonsentrasie, en ‘n volledige lipiedprofiel te bepaal. Geselekteerde rooibloedselmembraan VSe en VS verhoudings is bepaal, terwyl daar van ‘n ensiemgekoppelde immunosorberende toets (ELISA) gebruik gemaak is om serum insulienagtige groeifaktor-1 (IGF-1) en leptienkonsentrasies te bepaal. Antropometriese metings en bio-elektrieses impedansie analises (BEI) is ook uitgevoer.

Resultate: Die voorkoms van die MetS was 42.5 %, waarvan abdominale vetsug (100.0 % vir die MetS, en 39.1 % vir die nie-MetS), hipertensie (82.4 % vir die MetS, en 47.8 % vir die nie-MetS), en lae hoë-digtheidlipoproteïencholesterol (HDL-c) (76.5 % vir die MetS, en 34.8 % vir die nie-MetS) die mees algemene MetS risikofaktore was. Verskeie statistiesbetekenisvolle verskille is tussen die MetS en die nie-MetS groepe vir bloedparameters, insluitend insulien en HDL-c vlakke ($p<0.001$), glukose, IGF-1, en leptien vlakke ($p<0.05$) waargeneem. Die MetS groep het ook betekensivolle hoër antropometriese metings opgelewer, insluitend LMI ($p<0.05$), middelomtrek (MO), middel-heup verhouding (M:H) en sagitale abdominale deursnit (SAD) (almal $p<0.001$). Verder is betekenisvolle verskille in BEI (insluitend viserale vetweefsel (VVW) area, persentasie VVW (VVW %), subkutane vetweefsel (SVW %), en VVW:SVW verhouding ($p<0.001$ vir almal) tussen die MetS en nie-MetS groepe waargeneem. Geen betekensvolle verskil vir die individuele VSe of VS verhoudings is gevind nie. Klassifisering volgens die metaboliese status en LMI, het verskeie metabolies-geassosieerde parameters, antropometriese metings, en BEI voorspel. Die metaboliese status en LMI het nie die individuele VSe of VS verhoudings beïnvloed nie. Die vetsugtige MetS groep het verhoogde IGF-1 vlakke teenoor normale gewig, nie-MetS individue gelewer. Verskeie antropometriese metings, BEI, VSe en metabolies-gessosiëerde bloedmetings het korrelasies getoon.

Gevolgtrekking: Die resultate van hierdie studie stel voor dat die metaboliese status, en die gekombineerde effek van die metaboliese status en die LMI verandering in die metabolies-geassosieerde bloedparameters, antropometriese metings en BEI, in vroue kan voorspel. Dit kan moontlik verdere vetsugtigheid in die MetS met ‘n verhoogde risiko vir die ontwikkeling van lewenstyl-geassosieerde kanker verbind.

Sleutelwoorde:
Metaboliese sindroom, vetsuurprofiel, liggaamsamestelling, leptien, kankerrisiko

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LIST OF SYMBOLS

-	to
%	percentage
&	and
$\frac{1}{2}$	half
$\frac{1}{4}$	one-quarter
$\frac{3}{4}$	three-quarters
/	or
:	to
@	at
~	approximately
<	smaller than
=	equal to
>	greater than
±	approximately
÷	divided by
≤	equal to or smaller than
≥	equal to or greater than
↑	increase
↓	decrease
©	copyright
°	degrees
μ	micro
♀	female
♂	male
C	Celcius
κ	kappa
™	trademark
x	times
α	alpha
β	beta
Δ	delta
σ	sigma
Ω	omega

LIST OF ABBREVIATIONS

A

AA	arachidonic acid (20:4n-6)
AHA/NLBI	American Heart Association/National Heart, Lung and Blood Institute
ANOVA	analysis of variance
Apo B	apolipoprotein B
ATP III	National Cholesterol Education Program's Adult Treatment Panel III Report
α-LA	α-linolenic acid (18:3n-3)

B

b/m	beats per minute
BIA	bioelectrical impedance analysis
BMI	body mass index
BP	blood pressure

C

CANSA	Cancer Association of South Africa
cm	centimetre
cm^2	square centimetre
CNS	central nervous system
CPUT	Cape Peninsula University of Technology
CT	computed tomography
CVD	cardiovascular disease

D

d	distance
DBP	diastolic blood pressure
DGLA	dihomo-γ-linolenic acid (20:3n-6)
dH_2O	distilled water

DHA	docosahexaenoic acid (22:6n-3)
DPA	docosapentaenoic acid (22:5n-3)
Δ-9 SCD 1	Δ-9 stearoyl-CoA desaturase 1 enzyme
Δ-9 SCD 2	Δ-9 stearoyl-CoA desaturase 2 enzyme

E

EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
EPA	eicosapentaenoic acid (20:5n-3)

F

FA	fatty acid
FAME	fatty acid methyl esters
FBG	fasting blood glucose
FFA	free fatty acids
FFQ	food frequency questionnaire

G

g	gram
GC	gas chromatography
GH	growth hormone
GLC	gas liquid chromatography
GPAQ	global physical activity questionnaire

H

H	heaped
HC	hip circumference
HDL-c	high density lipoprotein cholesterol
HIV/AIDS	human immunodeficiency virus/acquired immunodeficiency syndrome
HOMA-IR	homeostasis model of insulin resistance
hr	hour
HREC	Human Research Ethics Committee

HRP	horseradish peroxidase
hsCRP	high-sensitivity C-reactive protein

I

IDF	International Diabetes Federation
IGF-1	insulin-like growth factor-1
IGF-1/IR-A	insulin-like growth factor-1/insulin receptor-A heterodimer
IGF-1R	insulin-like growth factor-1 receptor
IGFBPs	insulin-like growth factor binding proteins
IL-1β	interleukin-1β
IL-6	interleukin-6
IR	insulin resistance
ISAK	International Society for Advancement of Kinanthropometry

J

JIS	Joint Interim Statement

K

kg	kilogram
kg/m^2	kilograms per square metre
kHz	kilohertz

L

L	litre
LA	linoleic acid (18:2n-6)
LDL-c	low density lipoprotein cholesterol
LPL	lipoprotein lipase
Ltd	limited

M

µg/min	micrograms per minute
µL	microlitre
m	metre
MetS	metabolic syndrome
MetS+	with metabolic syndrome
MetS-	without metabolic syndrome
mg/g	milligrams per gram
mIU/L	milli-international units per litre
mL	millilitre
mmHg	millimetre of mercury
mmol/L	millimol per litre
MRC	Medical Research Council
MRI	magnetic resonance imaging
MUFA	monounsaturated fatty acids

N

NCDs	non-communicable diseases
NCEP	National Cholesterol Education Program
ng/mL	nanogram per millilitre
NHANES	National Health and Nutrition Examination Survey
NHLS	National Health Laboratory Service
nm	nanometre
NMetS-	normal weight without the MetS
NO	nitric oxide
non-MetS	without the metabolic syndrome

O

Ω-3:Ω-6	Ω-3 to Ω-6 FA ratio
OA	oleic acid (18:1n-9)
OBMetS+	obese with the MetS
OBMetS-	obese without the MetS
OD	optical density
OWMetS+	overweight with the MetS

OWMetS-	overweight without the MetS

P

PA	palmitic acid (16:0)
pg/mL	picogram per millilitre
PLA	palmitoleic acid (16:1n-7)
PUFA	polyunsaturated fatty acids

R

RAAS	renin angiotensin-aldosterone system
RBC	red blood cell
rpm	revolutions per minute
RT	room temperature

S

SA	stearic acid (18:0)
SA:OA	stearic acid to oleic acid ratio
SAD	sagittal abdominal diameter
SAT	subcutaneous adipose tissue
SAT %	percentage subcutaneous adipose tissue
SBP	systolic blood pressure
SCD	stearoyl-CoA desaturase
SEM	standard error of the mean
SFA	saturated fatty acids
SI	saturation index
SSA	sub-Saharan Africa
SST	serum separating tube

T

T2DM	type 2 diabetes mellitus
TB	tuberculosis
TEM	technical error of measurement
TG	triglyceride

TMB	3,3',5,5'-tetramethylbenzidine
TNF-α	tumour necrosis factor-α

V

v/v	volume per volume
VAT	visceral adipose tissue
VAT %	percentage visceral adipose tissue
VAT:SAT	visceral- to subcutaneous adipose tissue ratio
VLDL	very low density lipoprotein
VLDL-c	very low density lipoprotein cholesterol
vs	*versus*

W

W:H	waist-to-hip ratio
W:Ht	weight-to-height ratio
WAT	white adipose tissue
WBC	white blood cell
WC	waist circumference
WCRFI	World Cancer Research Fund International
WHO	World Health Organisation

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Recently, considerable focus was placed on non-communicable diseases (NCDs) as the leading cause of death worldwide, with an estimated 73.0 % of deaths attributed to this in low- and middle income countries (WHO, 2014a). Metabolic diseases form part of these NCDs, and are also increasing globally, resulting in the emergence of cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), and lifestyle-associated cancers (WHO, 2013). It has further been shown that a health transition is currently taking place in sub-Saharan Africa (SSA), which escalates disease burden even more (Young *et al.*, 2009; WHO, 2010a). A recent study ascribed the association of the increased prevalence of obesity, T2DM, CVD, and lifestyle-associated cancers in SSA with mortality (approximately 25.0 %), due to urbanisation (Steyn *et al.*, 2012; WHO, 2014a). Several factors may be dominant in this circumstance, including environmental, genetic, and lifestyle factors (Steyn *et al.*, 2012). These factors are interrelated, leading to a combination of metabolic-associated changes, such as abdominal obesity, hypertension, hyperglycaemia, insulin resistance (IR), and dyslipidaemia, collectively known as the metabolic syndrome (MetS) (Figure 1.1) (WHO, 2005; Alberti *et al.*, 2009; Jeon *et al.*, 2011; Erasmus *et al.*, 2012; Klug *et al.*, 2012; Camargo *et al.*, 2014; WCRFI, 2014).

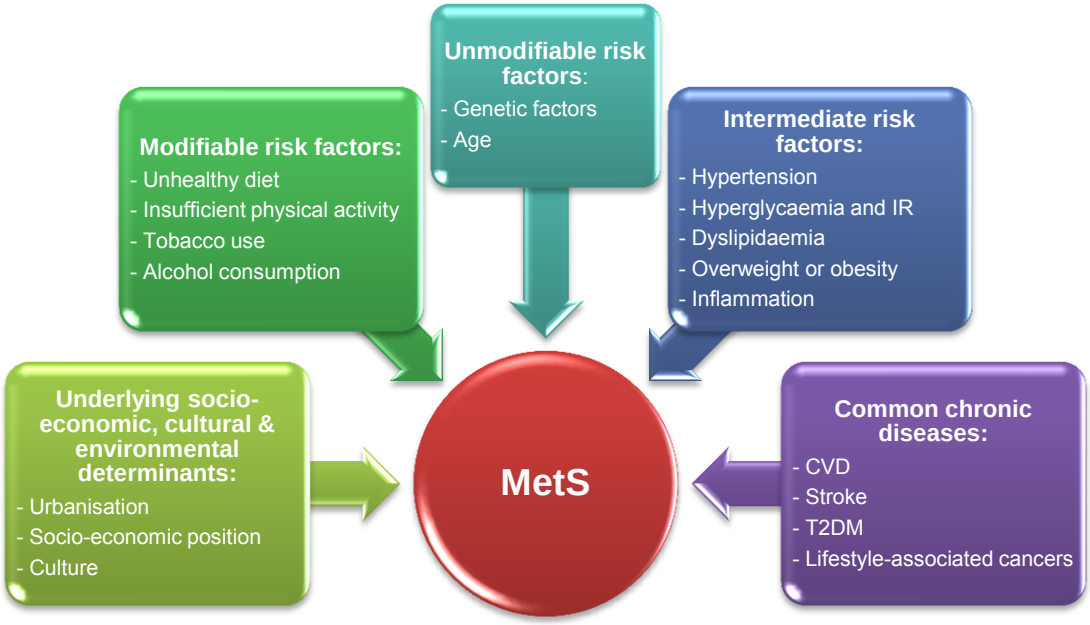

Figure 1.1: Common causes and risk factors of chronic non-communicable diseases.
(Adapted from: WHO, 2005; Park *et al.*, 2012; Peer *et al.*, 2013; Pradeilles *et al.*, 2015).

The MetS is associated with alterations in lipid metabolism, specifically dysregulation of fatty acid (FA) metabolism (Kabagambe *et al.*, 2008; Patel *et al.*, 2010; Novgordtseva *et al.*, 2011; Shab-Bidar *et al.*, 2014). This has led to the assumption that visceral adiposity and metabolic derangements were linked to significant correlations between blood FA concentrations in individuals with the MetS and healthy controls (Kabagambe *et al.*, 2008; Novgorodtseva *et al.*, 2011; Zhang *et al.*, 2012a; Zong *et al.*, 2013; Žák *et al.*, 2014). Moreover, individuals with the MetS showed significantly higher plasma saturated FA (SFA) and monounsaturated FA (MUFA) levels, and lower polyunsaturated FA (PUFA) levels compared to healthier controls (Kawashima *et al.*, 2009; Žák *et al.*, 2014).

The MetS is also associated with an increased risk of several lifestyle-associated cancers, including thyroid, oesophageal, colorectal, postmenopausal breast, urinary bladder, and cancer of the central nervous system (CNS) (Kuchiba *et al.,* 2012; Leiba *et al.*, 2012; Reeves *et al.*, 2012; Kawai *et al.*, 2013; Wiedman *et al.*, 2013; Li *et al.*, 2014; Lindkvist *et al.*, 2014; Roswall *et al.*, 2014; Almquist *et al.*, 2015). Studies highlighted elevated body mass index (BMI), the use of alcohol and/or tobacco, physical inactivity, and reduced intake of fruits and vegetables as some of the lifestyle factors that may be responsible for the increased risk of developing NCDs (Lindkvist *et al.*, 2014; Almquist *et al.*, 2015; Miglani *et al.*, 2015; WHO, 2015a).

This chapter provides background on the burden of the MetS, the different definitions and classifications used, with a specific focus on the South African setting. Furthermore, this chapter will explore the effects of the MetS and its association with different FAs, and cancer risk. This chapter concludes with the significance, aim, and objectives of the study.

1.2 THE METABOLIC SYNDROME (MetS)

1.2.1 Introduction

The MetS is a cluster of risk factors that are mostly associated with abdominal obesity or IR/diabetes (Alberti *et al.*, 2006; IDF, 2006). Although various definitions exist to diagnose the MetS, there is currently no standardised internationally accepted version of classifying specific populations. This confounds the comparison of different studies worldwide, since the prevalence of the MetS is dependent on the definition being used.

1.2.2 Definitions and classifications of the metabolic syndrome

The World Health Organisation (WHO) formulated the initial MetS definition in 1998. Here, IR was considered essential for the diagnosis of the MetS, along with any two additional risk factors, including obesity, hypertension, elevated triglycerides (TGs), reduced high density

lipoprotein cholesterol (HDL-c), or microalbuminuria (WHO, 1999; Alberti *et al.*, 2009). This definition was also the first to include microalbuminuria as a MetS risk factor (WHO, 1999).

The National Cholesterol Education Program Adult Treatment Panel III (ATP III) formulated a definition of the MetS stating that neither IR nor any other single risk factor was essential, although the presence of any three of the known MetS risk factors (obesity, hypertension, hyperglycaemia or dyslipidaemia) was needed (NCEP, 2001). The International Diabetes Federation (IDF) uses abdominal obesity, and any two risk factors to define the MetS (IDF, 2006). This definition also made use of similar cut-off values proposed by the ATP III definition (NCEP, 2001). Although obesity is associated with several metabolic risk factors (including hypertension, IR, hyperglycaemia, and dyslipidaemia), only the IDF requires abdominal obesity as essential for MetS diagnosis (IDF, 2006). Furthermore, this definition also specifically defined different cut-off values for abdominal obesity in different ethnic groups (summarised in Table 1.1) (IDF, 2006).

Table 1.1: Waist circumference cut-off values for different ethnic groups according to the IDF.

COUNTRY/ETHNIC GROUP	WC CUT-OFF VALUES	
	Males	**Females**
Europeans *	≥94.00 cm	≥80.00 cm
South Asians **	≥90.00 cm	≥80.00 cm
Chinese	≥90.00 cm	≥80.00 cm
Japanese ***	≥90.00 cm	≥80.00 cm
Ethnic South and Central Americans	Use South Asian recommendations until more specific data are available	
Sub-Saharan Africa	Use European data until more specific data are available	
Eastern Mediterranean and Middle East populations	Use European data until more specific data are available	

* In future epidemiologic studies of populations of European origin, prevalence should be given using both European and North American cut-points to allow better comparisons.
** Based on a Chinese, Malay and Asian-Indian population.
*** Originally different values were proposed for Japanese people, but new data support the use of the values shown above.
(IDF, 2006).

In an attempt to resolve the use of different definitions of the MetS, the IDF and the American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI), suggested that abdominal obesity should be omitted as an essential risk factor for MetS diagnosis (Alberti *et al.*, 2009). Instead, the resultant Joint Interim Statement (JIS) deemed the use of any three of the five risk factors as sufficient. The JIS definition is therefore almost similar to that of the IDF, except that abdominal obesity is not an essential risk factor (IDF, 2006; Alberti *et al.*, 2009). The different MetS definitions are summarised in Table 1.2.

Table 1.2: Different clinical definitions of the metabolic syndrome.

	WHO	ATP III	IDF	JIS*
Reference	(WHO, 1999)	(NCEP, 2001)	(IDF, 2006)	(Alberti *et al.*, 2009)
Criteria	IR diagnosed as T2DM, Impaired glucose regulation or ≥2 factors of the following:	≥3 of the following:	Increased WC according to ethnic group** and ≥2 of the following:	≥3 of the following:
Abdominal obesity	W:H ≥0.90 (men) ≥0.85 (women) or BMI≥30.00 kg/m^2	Men: WC ≥102.00 cm Women: ≥88.00 cm	Men: WC ≥94.00 cm Women: ≥80.00 cm**	Men: WC ≥90.00 cm Women: ≥80.00 cm
Impaired glucose regulation	Fasting plasma glucose ≥6.10 mmol/L 2hr post-glucose load ≥7.80 mmol/L	Fasting plasma ≥6.10 mmol/L	Fasting plasma ≥5.60 mmol/L Or T2DM diagnosis	Fasting plasma ≥5.60 mmol/L Or treatment for elevated glucose
Hyperinsulinaemia	≥1.70 mmol/L			
TG	≥1.70 mmol/L	≥1.70 mmol/L	≥1.70 mmol/L	≥1.70 mmol/L Or treatment for elevated TG
HDL-c	Men: <0.90 mmol/L Women: <1.00 mmol/L Or treatment for reduced HDL-c levels	Men: <1.03 mmol/L Women: <1.29 mmol/L	Men: <1.03 mmol/L Women: <1.29 mmol/L Or treatment for reduced HDL-c	Men: <1.03 mmol/L Women: <1.29 mmol/L Or treatment for reduced HDL-c
Hypertension	BP ≥140.00/≥90.00 mmHg Or treatment for hypertension	BP ≥130.00/≥85.00 mmHg Or treatment for hypertension	BP ≥130.00/≥85.00 mmHg Or treatment for hypertension	BP ≥130.00/≥85.00 mmHg Or treatment for hypertension
Microalbuminuria	Urinary albumin excretion rate ≥20.00 µg/min Albumin/Creatinine ratio ≥30.00 mg/g	-	-	-

* Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society and International Association for the study of obesity.

** Different cut-off values for different ethnic groups, including Europids, South Asians, Chinese, and Sub-Saharan Africans. The latter group uses the European cut-off values until specific data become available.

Public health studies originating from South Africa mainly used the IDF's definition (Jennings *et al.*, 2009; Erasmus *et al.*, 2012). As illustrated in Table 1.2, the IDF definition includes abdominal obesity (as measured by waist circumference (WC)) in combination with any two other MetS-specific criteria (IDF, 2006). It is therefore expected that the prevalence of the MetS will differ between developed and developing countries, since different MetS definitions use different criteria and cut-off values.

1.2.3 Prevalence of the metabolic syndrome

1.2.3.1 Global vs local

The estimated global MetS prevalence ranged between 20.0-25.0 % according to the IDF in 2006 (IDF, 2006). African studies reported this prevalence to vary as a function of geographical location and ethnicity. For example, the MetS prevalence in Nigeria and Ghana vary between 12.1 % and 35.9 % respectively (Adegoke *et al.*, 2010; Gyakobo *et al.*, 2012), whereas a relatively recent South African study reported a much higher prevalence of 42.1 % (Crowther & Norris, 2012).

Various studies have shown the prevalence of the MetS to be more associated with women (Garrido *et al.*, 2009; Motala *et al.*, 2011; Erasmus *et al.*, 2012; Gyakobo *et al.*, 2012). Motala *et al.* (2011) reported an overall prevalence of 23.3 % amongst a population in Kwa-Zulu Natal, with women displaying more than double the prevalence (26.3 % *vs* 11.6 %, IDF definition). More recently, a South African study amongst a coloured population in the greater Cape Town region, reported that 30.6 % of men and almost 70.0 % of women older than 31 years, were classified as having the MetS (Erasmus *et al.*, 2012). In contrast, Hoebel *et al.* (2013) noted a much higher MetS prevalence amongst Black and Caucasian men (59.0-77.0 % in men *vs* 22.0-69.0 % in women) between the ages of 25 and 65 years from an urban environment. The latter study concluded that the higher MetS prevalence in men was due to the level of urbanisation and associated lifestyle choices such as increased alcohol intake and tobacco use, unhealthy dietary habits, and physical inactivity (Hoebel *et al.*, 2013). Furthermore, gender and ethnic disparities in lipid and glucose metabolism may contribute to variations in the prevalence of the MetS and its individual risk components (Hoebel *et al.*, 2013; Rochlani *et al.*, 2015).

1.2.3.2 Prevalence of the individual components of the metabolic syndrome

Several studies reported contrasting results regarding the most and least prevalent individual MetS risk factor for different populations. Garrido *et al.* (2009) observed low HDL-c levels as the most prevalent MetS risk factor, although several more recent studies established that abdominal obesity was more prevalent (Hoebel *et al.,* 2011; Gyakobo *et al.,* 2012; Peer *et al.*, 2015; Salonen *et al.*, 2015; Strand *et al.,* 2015). According to Hoebel *et al.* (2011) elevated fasting blood glucose (FBG), low HDL-c levels, and hypertension were the second, third and fourth most common individual MetS risk factor respectively, while a very low prevalence of elevated TG levels was noted. Similarly, Gyakobo *et al.* (2012), Peer *et al.* (2015) and Salonen *et al.* (2015) also found low HDL-c and hypertension to be the second and third most prevalent individual MetS risk factor, respectively. Strand *et al.* (2015) reported that hypertension was the

second most prevalent MetS component, followed by low HDL-c and high TG levels, while very few participants presented with elevated FBG levels. Although most studies have shown consensus regarding the prevalence of the individual components of the MetS, the order in which these factors appear largely depends on the population being studied, as well as the definition used.

Abdominal obesity

Obesity is regarded as a state of excessive fat accumulation to such an extent that general health is impaired (WHO, 2015b). The global prevalence of adult overweight or obesity was estimated to be approximately 2.1 billion (Ng *et al.*, 2014), which is particularly alarming, since it is also estimated that the rising obesity rates in children will contribute to the increase in global obesity prevalence during adulthood (Abrahams *et al.*, 2011; Steyn *et al.*, 2012).

Globally, the prevalence of adult overweight and obesity increased by nearly 30.0 % in three decades, independent of gender or ethnicity (Ng *et al.*, 2014). In contrast to the data from developed countries, female obesity is on the rise in many low- and middle income countries (Garrido *et al.*, 2009; Alkerwi *et al.*, 2011; Crowther & Norris, 2012; Erasmus *et al.*, 2012; Peer *et al.*, 2013; Senekal *et al.*, 2015). Garrido *et al.* (2009) reported that 27.3 % and 28.7 % of the Batswanas were overweight and obese, which are significantly lower than the nearly 64.0 % of sub-Saharan African women that were overweight in 2014 (Ng *et al.*, 2014). Furthermore, this study showed that specifically in South African women, the prevalence of being overweight was much higher than the prevalence of obesity (69.3 % *vs* 42.0 %) (Ng *et al.*, 2014).

With specific reference to using WC as the anthropometric measure of abdominal obesity, Motala *et al.* (2011) found that 96.9 % of women presented with abdominal obesity. The prevalence of abdominal obesity (WC≥80 cm) reported here, was higher than that reported in other African countries, including Botswana (42.0 %), and Ghana (55.3 %) (Garrido *et al.*, 2009; Gyakobo *et al.*, 2012). Particularly in South Africa, more Caucasian women presented with abdominal obesity compared to Black women (77.9 % *vs* 75.6 %) (Hoebel *et al.*, 2011; Crowther & Norris, 2012).

Studies have also indicated that measures of obesity differ between MetS and non-MetS groups. Here, MetS participants presented with significantly higher BMIs and WCs compared to their non-MetS counterparts (Nigam *et al.*, 2009; Bremer *et al.*; 2011; Esteghamati *et al.*, 2011; Atanassova *et al.*, 2014). Studies have also shown that obesity is strongly associated with hypertension (Wildman *et al.*, 2008; Jeon *et al.*, 2011; Peer *et al.*, 2013; Abu-Farha *et al.*, 2014; Helelo *et al.*, 2014), since the relationship between obesity and microvascular dysfunction and

abnormal vascular tone increases vasoconstriction, and leads to elevated BP (de Boer *et al.*, 2011; Peer *et al.*, 2013, Helelo *et al.*, 2014; Kaur, 2014).

Hypertension

Hypertension is defined as an elevated systolic blood pressure (SBP) of more than 130 mmHg, and a diastolic blood pressure (DBP) of more than 85 mmHg (IDF, 2006). Hypertension is not only globally on the increase (80 million in 2000 to 150 million by 2025) (WHO, 2014b), but is also considered a major public health challenge in Africa. For example, Garrido *et al.* (2009) reported that 44.0 % of the Batswanas presented with hypertension, while only 22.4 % of Ethiopia's population were hypertensive (Helelo *et al.*, 2014). According to the WHO, almost a third of the men and women in Africa presented with hypertension in 2014 (WHO, 2014b), whilst more than 20.0 % of adults suffered from hypertension in 2015 worldwide (WHO, 2015c).

In South Africa specifically, earlier studies reported a hypertension prevalence of 36.4 % in apparently healthy Black women, and an almost 40.0 % prevalence in participants in Cape Town (Crowther & Norris, 2012; Peer *et al.*, 2013). This is lower than the nearly 50.0 % hypertension prevalence reported for Black women in more recent studies from the Western Cape (Peer *et al.*, 2015; Senekal *et al.,* 2015). Several confounding factors such as ethnicity, familial hypertension history, lifestyle factors, and access to healthcare may contribute to the prevalence of hypertension (Umscheid *et al.*, 2010; Jeon *et al.*, 2011; Peer *et al.*, 2013; Helelo *et al.*, 2014; Kaur, 2014; Rochlani *et al.*, 2015). Studies have also confirmed that sex hormones are associated with hypertension. Here, oestrogen plays a protective role by regulating the renin angiotensin-aldosterone system (RAAS) through increased angiotensinogen, and decreased renin and angiotensin converting enzyme synthesis (Denton *et al.*, 2013; Hilliard *et al.*, 2013; Rochlani *et al.*, 2015). However, during ageing, the protective effects of oestrogen on hypertension are reduced, leading to the increased prevalence of hypertension among postmenopausal women (Denton *et al.*, 2013; Hilliard *et al.*, 2013; Rochlani *et al.*, 2015). Additionally, ageing is also associated with hypertension due to arterial thickening and increased arterial resistance (Helelo *et al.*, 2014).

Increased stress levels and urbanisation also contribute to hypertension (Jeon *et al.*, 2011; Peer *et al.*, 2013; Helelo *et al.*, 2014; Rochlani *et al.*, 2015). Poor lifestyle choices, such as high salt intake and processed foods are associated with hypertension, because increased salt intake disrupts sodium homeostasis, causing fluid retention, thereby increasing BP (Sheng, 2009; Helelo *et al.*, 2014). Hypertension is also associated with other lifestyle factors such as physical inactivity, smoking, and alcohol consumption (Helelo *et al.*, 2014). Furthermore, obesity, adipose tissue morphology and -distribution, IR, glucose intolerance/diabetes, and dyslipidaemia may

also affect BP (Umscheid *et al.,* 2010; Jeon *et al*., 2011; Peer *et al*., 2013; Kaur, 2014). Since these factors are related to the MetS, it is not surprising that SBP and DBP were significantly higher in individuals with the MetS (Nigam *et al.*, 2009; Bremer *et al.,* 2011; Esteghamati *et al.*, 2011). Hypertension is also associated with glucose intolerance and IR, since hyperinsulinaemia increases sodium resorption and leads to vasodilation and volume expansion, and ultimately increases BP (Alberti *et al.*, 2006; Kirk & Klein, 2009; Jennings *et al*., 2009).

Glucose intolerance and insulin resistance

It is well known that individuals with IR not only present with impaired glucose metabolism, but also elevated FBG, hyperglycaemia, reduced glucose clearance due to decreased insulin activity, and reduced suppression of glucose production (Kaur, 2014). The prevalence of elevated FBG differed between African countries, and ranged from 12.5 % (Ghana) to 26.7 % (Botswana) (Garrido *et al.,* 2009; Gyakobo *et al.*, 2012). Several South African studies amongst Black and Caucasian women noted that 20.0-25.0 % of participants presented with elevated FBG levels (Crowther & Norris 2012; Peer *et al.*, 2015; Senekal *et al.*, 2015). Evans *et al.* (2011) reported clear ethnic differences, where Caucasian women had significantly higher FBG, and lower insulin levels compared to Black women. This is possibly due to ethnic differences in adipose tissue distribution, where Black women accumulate less visceral adipose tissue (VAT) with increasing WC (Evans *et al.*, 2011). Additionally, insulin sensitivity is gender-dependent, possibly due to differences in hormonal and adipose tissue distribution – women normally present with a higher body fat percentage, total adiposity, and insulin sensitivity (Rochlani *et al.*, 2015).

Several studies also found significantly elevated levels of FBG and insulin, and homeostasis in a model of insulin resistance (HOMA-IR) in participants with the MetS (Nigam *et al.*, 2009; Bremer *et al.,* 2011; Esteghamati *et al.*, 2011; Atanassova *et al.*, 2014). Additionally, glucose abnormalities have also been associated with dyslipidaemia, since IR has been correlated with elevated TG levels, possibly due to impaired enzyme activity (Sumner *et al*., 2005; Ginsberg & Karmally, 2009; Jennings *et al*., 2009).

Dyslipidaemia

Elevated, or reduced levels of the different blood lipid fractions are used to characterise dyslipidaemia, including elevated TG, low density lipoprotein cholesterol (LDL-c), and reduced HDL-c levels (WHO, 1999; NCEP, 2001; IDF, 2006; Alberti *et al.*, 2009). Low HDL-c levels were common in some African countries, including Botswana (80.0 %), South Africa (45.0 %), and Ghana (42.7 %) (Garrido *et al.*, 2009; Evans *et al.*, 2011; Gyakobo *et al.*, 2012). Crowther & Norris (2012) reported a prevalence of 70.1 % in their South African population for reduced

HDL-c levels, and 22.3 % exhibited elevated TG levels. In accordance, Crowther & Norris (2012) and Peer *et al.* (2015) also found that low HDL-c levels were more common than elevated TG levels in South African women. Regarding ethnicity, an earlier South African study found that 70.5 % of Black women presented with low HDL-c levels, while elevated TG levels were uncommon in this population (prevalence of ~8.0 %) (Hoebel *et al.*, 2011). This is in contrast to the study of Evans *et al.* (2011) in which ethnicity affected all areas of the lipid profile, with Caucasian South African women displaying higher total cholesterol, TG, and HDL-c levels, compared to their Black counterparts. A recent study by Senekal *et al.* (2015) reported that a third of a South African study population presented with elevated total cholesterol levels. The discrepancies in the prevalence reported could be ascribed to several factors, such as grade of obesity, gender, ethnicity, and lifestyle factors (Evans *et al.,* 2011; Delisle *et al.*, 2013). These findings could also reflect the effects of inflammation, and environmental factors on the lipid profile (Evans *et al.*, 2011).

It is also well established that the MetS is associated with dyslipidaemia (IDF, 2006; Shab-Bidar *et al.*, 2014). Total cholesterol, LDL-c, and TG levels were all significantly elevated in MetS individuals; whereas HDL-c levels were significantly lower (Nigam *et al.*, 2009; Bremer *et al.,* 2011; Esteghamati *et al.*, 2011; Atanassova *et al.*, 2014). Lipid profiles of normal and obese individuals indicated that obesity was a major risk factor for both dyslipidaemia and the MetS. This was shown in two different studies, in which obese participants had significantly higher total cholesterol, LDL-c and TG levels, and significantly lower HDL-c levels compared to normal weight controls (Jeon *et al.*, 2011; Gómez-Ambrosi *et al.*, 2012). All the individual components of the MetS can also lead to several pathologies in different physiological systems (Alberti *et al.*, 2006; Jeon *et al.*, 2011; Crowther & Norris, 2012; Camargo *et al.*, 2014).

1.3 PATHOPHYSIOLOGY OF THE METABOLIC SYNDROME

1.3.1 Introduction

The MetS is a complex of interconnected metabolic-related risk factors; with MetS patients suffering from IR, glucose intolerance, postprandial dyslipidaemia, and pro-inflammatory and pro-thrombotic states (Alberti *et al.*, 2006; Jeon *et al.*, 2011; Crowther & Norris, 2012; Camargo *et al.*, 2014). These alterations frequently lead to several other metabolic-related diseases, such as CVD, T2DM, and an increased risk for the development of lifestyle-associated cancer (Alberti *et al.*, 2009; Crowther & Norris, 2012; Erasmus *et al.*, 2012; Klug *et al.*, 2012; Lindkvist *et al.*, 2014; Roswall *et al.*, 2014; Almquist *et al.*, 2015). Although the pathophysiology of the MetS is multifaceted, abdominal fat distribution, and IR may be some of the more dominant risk factors (Alberti *et al.*, 2006; IDF, 2006; Jeon *et al.*, 2011; Crowther & Norris, 2012; Camargo *et al.*,

2014) (Figure 1.2). Insulin resistance refers to a state of reduced sensitivity to the effects of insulin, which is closely associated with obesity (Alberti *et al*., 2006; IDF, 2006; Jeon *et al*., 2011; Crowther & Norris, 2012; Camargo *et al*., 2014).

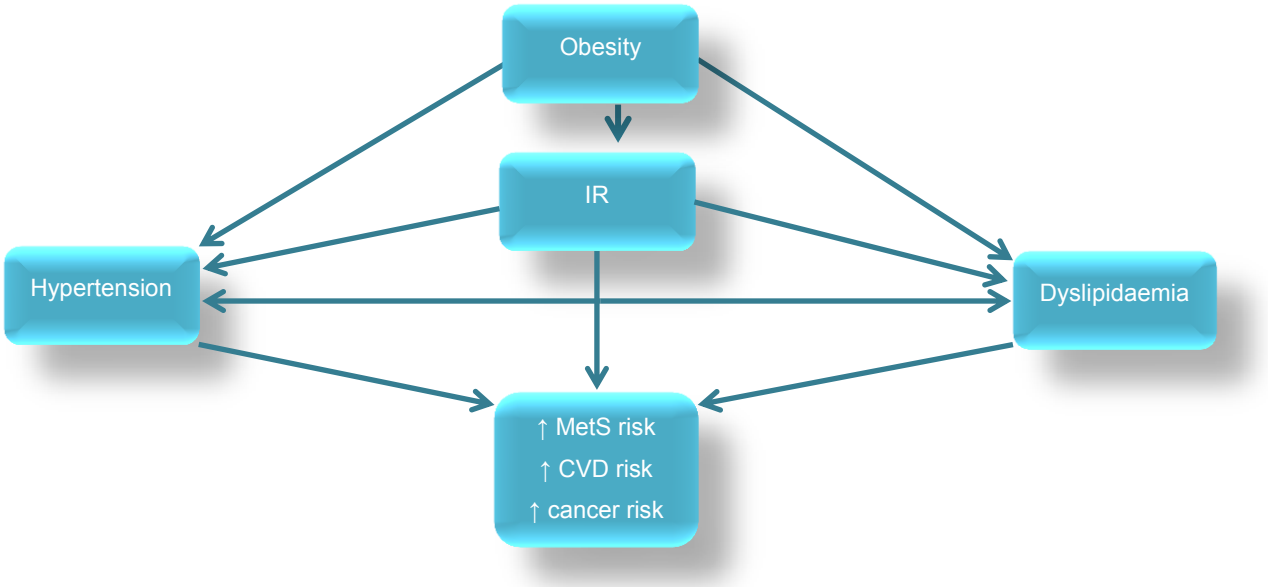

Figure 1.2: Outline of the key pathogenic factors involved in the pathophysiology of the metabolic syndrome, obesity and insulin resistance.
(Adapted from: Alberti *et al.*, 2006; IDF, 2006; Jeon *et al.*, 2011; Crowther & Norris, 2012; Camargo *et al.*, 2014).

1.3.2 Obesity

1.3.2.1 Introduction and health implications of obesity

Obesity is characterised by excessive lipid storage, and hence weight gain (Mendonça *et al.*, 2015; WHO, 2015b). Adipose tissue constitutes an estimated 15.0-25.0 % of body mass, and has several physiological functions including mechanical support, protection, thermal insulation, and energy storage (Levine & Levine, 2014).

In general, fat is stored as white adipose tissue (WAT), and can be further subdivided into (i) subcutaneous adipose tissue (SAT), and (ii) VAT, each with its own unique metabolic- and biochemical functions, and health risks (Shah *et al.*, 2012; Levine & Levine, 2014; Mendonça *et al.*, 2015; Yeoh *et al.*, 2015). Subcutaneous fat is located underneath the skin providing mechanical protection, and contribute to normal lipid- and glucose metabolism. The SAT deposition normally occurs in the gluteo-femoral region, and contributes to the gynoid-phenotype obesity, which is commonly seen in premenopausal women (Levine & Levine, 2014; Stewart & Sutton, 2012; Kranendonk *et al.*, 2015). The VAT compartment surrounds the internal organs, and secretes adipokines and cytokines. Visceral deposition normally occurs in the abdominal region and contributes to the android-phenotype obesity, which is more prevalent in

men (Levine & Levine, 2014; Stewart & Sutton, 2012; Kranendonk *et al.*, 2015). This gender-dependent fat distribution suggests that oestrogen plays an additive role, since SAT areas are more prominent in women, whereas VAT areas are greater in men (Camhi *et al.*, 2011; Schautz *et al.*, 2012; Shah *et al.*, 2012). Although adiposity differs between genders, ageing (and thus reduced oestrogen levels) was shown to contribute to android adiposity in menopausal women, leading to the disappearance of sexual dimorphisms (Alkerwi *et al.*, 2011; Camhi *et al.*, 2011).

The region of fat deposition is closely related to the associated metabolic dysfunction, and health implications, since metabolic dysfunction was related to VAT and SAT, with both components being positively associated with BMI (Pou *et al.*, 2009; Yim *et al.*, 2010; Camhi *et al.*, 2011; Shah *et al.*, 2014; Yeoh *et al.*, 2015). Individuals with a normal BMI tend to generally have lower VAT than those with a higher to overweight BMI (Shah *et al.*, 2014). Visceral adiposity is also positively associated with the MetS, even in people with a normal BMI, where they can exhibit higher visceral adiposity, higher FBG, as well as insulin levels. It is therefore suggested that individuals with a normal BMI, but greater visceral adiposity, have a biochemical phenotype similar to that of an overweight or obese person (Shah *et al.*, 2014). Recent studies found that an increasing VAT to SAT ratio (VAT:SAT) was associated with increasing CVD risk, which relates to the fact that VAT is regarded as more metabolically active, and therefore able to secrete larger quantities of pro-inflammatory adipokines compared to SAT (Mendonça *et al.*, 2015; Yeoh *et al.*, 2015). For this reason, visceral adiposity is more frequently related to metabolic alterations, such as IR, than subcutaneous adiposity (Shah *et al.*, 2012; Mendonça *et al.*, 2015).

Being overweight or obese are associated with adipose tissue dysfunction, which increases the risk of developing hypertension, dyslipidaemia, hyperglycaemia, CVD, T2DM, and certain lifestyle-associated cancers (Figure 1.3) (Jeon *et al.*, 2011; Gómez-Ambrosi *et al.*, 2012; Kuchiba *et al.*, 2012; Kawai *et al.*, 2013; Peer *et al.*, 2013; Helelo *et al.*, 2014; Lindkvist *et al.*, 2014; Almquist *et al.*, 2015). Increased VAT poses greater health risks due to its proximity to the intestines, since VAT facilitates energy storage, and is rich in parasympathetic innervation that upregulate visceral lipogenesis (Levine & Levine, 2014). The central location of VAT therefore makes it more susceptible to the effects of inflammatory and neuro-hormonal factors (Levine & Levine, 2014).

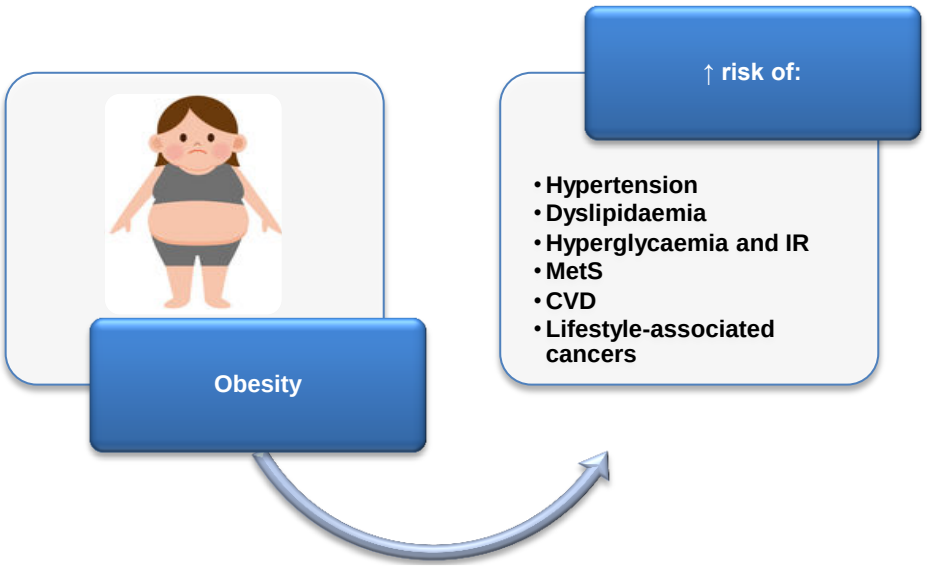

Figure 1.3: A brief outline of the pathophysiology of obesity.
(Adapted from: Jeon *et al.*, 2011; Gómez-Ambrosi *et al.*, 2012; Peer *et al.*, 2013; Helelo *et al.*, 2014).

Different anthropometric assessments are utilised to measure obesity as a risk factor for the MetS, and will be discussed in the following section (Anunciação *et al.*, 2014; Cheong *et al.*, 2015).

1.3.2.2 Abdominal obesity assessment methods

Several assessment methods exist to describe body composition and fat distribution (Willet & Hu, 2013). The most commonly used anthropometric measurements include body weight, BMI, skinfold thicknesses, and circumferences (NHANES, 2011). However, abdominal obesity is more easily and accurately assessed with the WC, waist-to-hip circumference ratio (W:H), waist-to-height ratio (W:Ht), or sagittal abdominal diameter (SAD), compared to using total body fat or the BMI (Anunciação *et al.*, 2014; Cheong *et al.*, 2015).

Body mass index and bioelectrical impedance analysis

The BMI (kg/m^2) accounts for differences in body composition by defining the level of adiposity according to the relationship of body weight and height (Willet & Hu, 2013). The BMI is calculated as the body weight (in kilograms) divided by the height (in metres) squared (Equation 1.1) (Jarvis, 2011; Willet & Hu, 2013).

$$Body\ mass\ index\ (kg/m^2) = \frac{Body\ weight\ (kg)}{Height\ (m)^2}$$

Equation 1.1: Calculation of body mass index.
(WHO, 2004a; NHANES, 2011).

The BMI is used to classify individuals as either underweight, normal, overweight or obese (summarised in Table 1.3) (Willet & Hu, 2013). Large epidemiological studies often use BMI to assess total body fat, and fat distribution, since it is a quick and easy measure (Willet & Hu, 2013).

Table 1.3: Classification of body mass index.

CLASS		BMI (kg/m^2)
Underweight	Severe malnutrition	<16.00
	Moderate malnutrition	16.00-16.99
	Mild malnutrition	17.00-18.49
Normal range	Normal	18.50-24.99
Overweight	Pre-obese	25.00-29.99
Obese	Obese class I	30.00-34.99
	Obese class II	35.00-39.99
	Obese class III	≥40.00

(WHO, 2004a; NHANES, 2011).

The specific ethnic, as well as gender cut-off values for BMI are also controversial, highlighting the need to establish population-, age-, and gender-specific cut-off values for different anthropometric measures (WHO, 2008; Stewart & Sutton, 2012). The use of the BMI has some disadvantages, including its inability to distinguish between excessive body weight due to fat- or muscle mass (Stewart & Sutton, 2012). Furthermore, the index does not reflect adipose tissue distribution, which is an important risk factor for several metabolic diseases (Willet & Hu, 2013).

Bioelectrical impedance analysis (BIA) is used to determine the distribution of body fat, and can therefore be used to assess VAT and SAT, and extra- and intracellular fluids (Stewart & Sutton, 2012). Here, electrodes are connected to the limbs of a participant to form a circuit for a small current to pass through to determine the body's impedance, since different tissue types vary in resistance to electric current due to differences in water content (Thibault *et al.*, 2012; Stewart & Sutton, 2012). For example, fat has a lower water content compared to muscle, which make fat a poor conductor with higher resistance than muscle (Santarpia *et al.*, 2009; Stewart & Sutton, 2012). Tissue reactance and -resistance are used to calculate the phase angle, where a smaller phase angle is associated with reduced cell membrane integrity and cell death, which is associated with the risk of disease (Santarpia *et al.*, 2009; Thibault & Pichard, 2012). The use of BIA is convenient in resource-limited settings, although several factors such as age, associated disease state (renal and liver disease for example), dehydration, pregnancy, and earlier vigorous exercise, should be considered when using this assessment method, since it may influence the accuracy of BIA measurements (Stewart & Sutton, 2012; Thibault & Pichard, 2012).

Some studies use the BMI in combination with BIA for more accurate estimations of the percentage storage and visceral fat. However, in some instances, BMI classification and BIA has led to diverse conclusions regarding obesity, which is possible since individuals with the same BMI class may have different body fat percentages, mainly influenced by factors such as ethnicity, gender, and age (Camhi *et al.*, 2011). Gómez-Ambrosi *et al.* (2012) reported that less than a third of individuals classified as having a normal BMI were actually obese according to body fat percentage. In addition, less than one percent of the study population classified as obese using the BMI, were actually classified as normal when they were assessed using percentage body fat (Gómez-Ambrosi *et al.*, 2012).

It is noteworthy to mention that, an individual with a normal BMI can be classified as 'metabolically healthy' or '-unhealthy' depending on the site of fat deposition (Wildman *et al.*, 2008). Increased adipose tissue storage in the visceral compartment is associated with an increased risk of the MetS, CVD, and cancer (Wildman *et al.*, 2008, Shapira, 2009). This is opposed to an obese person with predominant adipose tissue storage in the subcutaneous compartment, classified as 'obese and metabolically healthy', who is at a lower risk of developing the MetS, CVD, and cancer (Figure 1.4) (Wildman *et al.*, 2008). Therefore, misclassification of participants as overweight using the BMI when participants were actually obese according to body fat percentage can be misleading, hence emphasising the importance of using more than one measure of obesity assessment (Gómez-Ambrosi *et al.*, 2012).

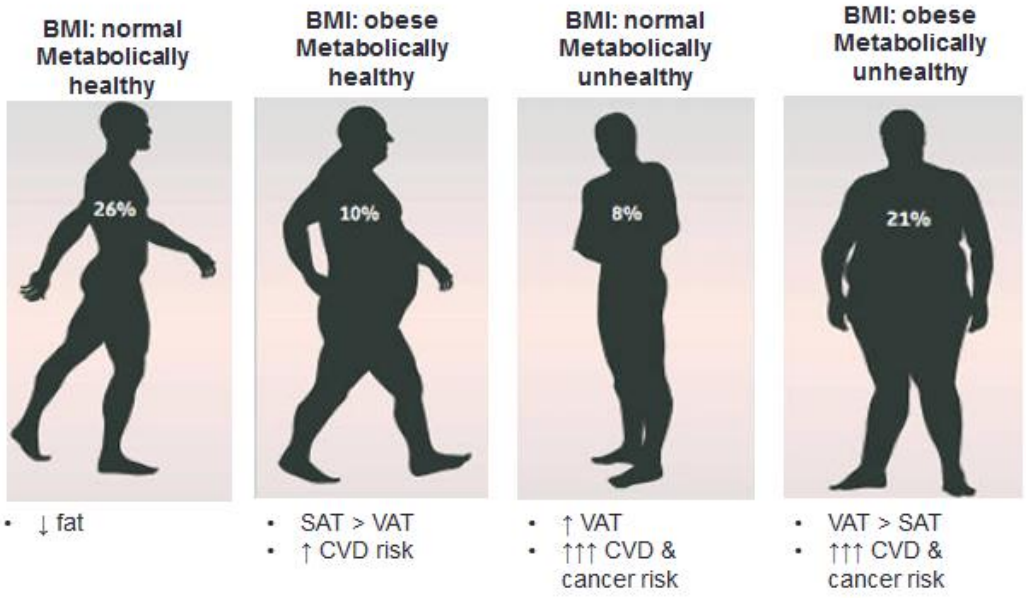

Figure 1.4: Metabolically healthy and -unhealthy, regardless of body mass index class.
(Adopted from: Wildman *et al.*, 2008).
* %=US adults (2008)

Waist circumference, hip circumference and other obesity assessment methods

Since BMI cannot accurately describe abdominal obesity, additional surrogate measures such as WC, hip circumference (HC), and the calculated W:H and W:Ht are used, because these have been shown to be better predictors of fat distribution (Anunciação *et al.*, 2014; Cheong *et al.*, 2015).

The WC and HC are used to indirectly assess abdominal adiposity, although these measurements may vary depending on several factors, such as time of day, standing position, posture, *etc*. (Willet & Hu, 2013). Strong correlations were evident between the WC, HC, and individual components of the MetS (Crowther & Norris, 2012; Cheong *et al.*, 2015). In order to classify individuals with the MetS, the IDF (2006) definition was proposed for SSA until further population-specific studies are available. Different cut-off values for WC have been identified for South African Caucasians (WC≤84.0 cm for women), and Africans (WC≤94.0 cm for women) (Hoebel *et al*., 2014), although very few studies have used these population-specific WC cut-off values.

Even though HC measurements are not routinely used, it is important in the calculation of the W:H, which estimates the risk for CVD (WC≥80.0 cm, HC≥88.0 cm or W:H≥0.85 for women) (IDF, 2006; WHO, 2008; Stewart & Sutton, 2012). With particular reference to disease risk, evidence suggests that the W:H is actually an inferior measure to identify individuals who are at risk for developing metabolic diseases (Evans *et al.*, 2011; Roswall *et al.*, 2014). Although the use of the WC, HC, and W:H is regarded as inferior measures of total body fat, these measures have a high capability of determining fat distribution (Willet & Hu, 2013). Abdominal obesity can also be assessed using the W:Ht, which is associated with cardiometabolic dysfunction (including dyslipidaemia, hypertension, and T2DM), despite normal BMI and WC measurements (Li *et al.*, 2011a; Ware *et al.*, 2014). A major advantage of the W:Ht compared to WC, is that this measure adjusts for height, and universally accepted cut-off values can be used for different ethnic groups (Li *et al.*, 2011a; Schneider *et al.*, 2011). Shorter people may present with a higher risk of developing metabolic abnormalities (30 %) compared to taller people (Schneider *et al.*, 2011).

Excessive abdominal fat (measured by WC, W:H, and/or W:Ht) is a major risk factor for disease development, including elevated levels of inflammatory markers, dyslipidaemia, IR, T2DM, cardiovascular and respiratory diseases, cancer, and ultimately mortality (Jacobs & Newton, 2010; Jarvis, 2011; Li *et al.*, 2011a; Ware *et al.*, 2014). Not only is a large WC associated with elevated levels of circulating inflammatory markers such as high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6) and interleukin-1β (IL-1β), it is also associated with increased

mortality in women, irrespective of their BMI (Jacobs & Newton, 2010; Bremer *et al.*, 2011). There is also a strong correlation between elevated W:H and an increased risk of several cancer types, including oesophageal- and colorectal cancer (Kendall *et al.*, 2013; Cottet *et al.*, 2015). Kendall *et al.* (2013) also reported a significant association between oesophageal cancer and another measure of abdominal obesity, the SAD.

Sagittal abdominal diameter

The SAD relies on the mid-sagittal plane to measure the linear distance across the abdomen, and is regarded a good measure of abdominal fat distribution to indirectly assess visceral adiposity (NHANES, 2011; Stewart & Sutton, 2012). Several metabolic alterations, such as lipid, insulin and glucose levels, and IR have been positively associated with the SAD (Kang *et al.*, 2010; Yamamoto *et al.*, 2010; Yim *et al.*, 2010; Anunciação *et al.*, 2014; Vasques *et al.*, 2015). Hypertension and the associated risk of CVD are also associated with SAD measurements (Yamamoto *et al.*, 2010; Carlsson *et al.*, 2014).

1.3.3 Hypertension

Hypertension, defined as both an elevated SBP higher than 130 mmHg, and a DBP of 85 mmHg, often leads to other diseases such as CVD, stroke, dementia, and renal failure (Helelo *et al.*, 2014; Lloyd-Sherlock *et al.*, 2014). Studies have shown that hypertension is associated with ageing, stress, urbanisation, dyslipidaemia, IR and glucose intolerance, as well as obesity (Jennings *et al*., 2009; Jeon *et al*., 2011; Peer *et al*., 2013; Kaur, 2014).

Obesity is associated with microvascular dysfunction, affecting blood flow resistance and tissue perfusion leading to hypertension (Alberti *et al.*, 2006; de Boer *et al.*, 2011). Hypertensive patients present with abnormal vasomotor tone that improves vasoconstriction or reduces vasodilation (de Boer *et al.*, 2011). Additionally, it was shown that adipocytes produce aldosterone in response to angiotensin II, which implies that adipocytes can affect BP by acting as small scale RAAS (Kaur, 2014). Furthermore, hypertension is also associated with other CVD risk factors, including significantly elevated total cholesterol, lower HDL-c levels, and abdominal obesity (Rochlani *et al.*, 2015).

Although the exact mechanism of hypertension in IR is still vague, activation of the sympathetic nervous system and RAAS are thought to play important roles (Jennings *et al.*, 2009; Kirk & Klein, 2009; Horita *et al.*, 2011; Sossa *et al.,* 2012; Kaur, 2014; Soleimani, 2015). Studies have shown that both hyperglycaemia and -insulinaemia activate the RAAS through increased expression of angiotensin II, leading to increased sodium resorption in the kidneys, increased cardiac output, vasoconstriction, and elevated BP (Kirk & Klein, 2009; Horita *et al.*, 2011; Kaur,

2014; O'Neill & O'Driscoll, 2015; Soleimani, 2015). Additionally, IR diminishes the vasodilatory effects of insulin, which leads to hypertension (Kirk & Klein, 2009; Horita *et al.*, 2011).

1.3.4 Glucose intolerance and insulin resistance

Fasting blood glucose levels higher than 5.6 mmol/L and insulin levels above 1.7 mmol/L are positively associated with impaired glucose regulation and hyperinsulinaemia (IDF, 2006). Normal insulin sensitivity is associated with normal body weight and the absence of abdominal obesity (Kaur, 2014). However, individuals with IR have impaired glucose uptake, which explains why blood glucose levels are elevated, and a normal insulin concentration does not lead to a normal insulin response (Kaur, 2014). Instead, more insulin is produced (causing a state of hyperinsulinaemia) to lower the elevated glucose levels (illustrated in Figure 1.5). (Jennings *et al.*, 2009; Sossa *et al.,* 2012; Mendonça *et al.*, 2015). Excessive insulin production may eventually impair the pancreatic β-cells to such a degree that an insufficient quantity of insulin are secreted, causing a state of hyperglycaemia, clinically diagnosed as T2DM (Jennings *et al.*, 2009; Sossa *et al.,* 2012).

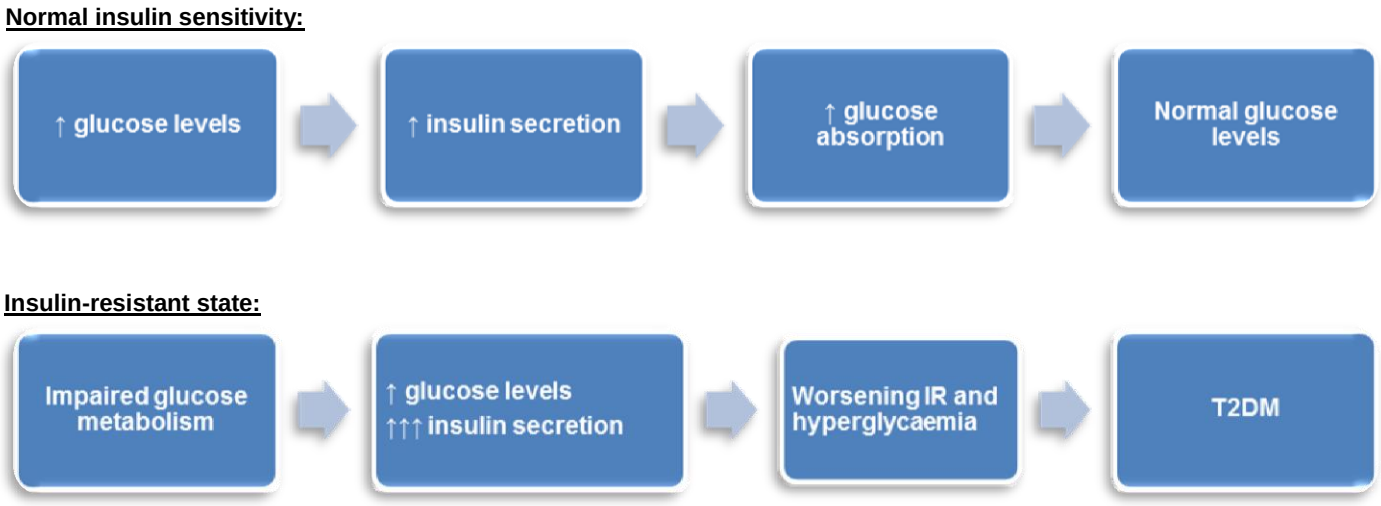

Figure 1.5: Schematic presentation of the health implications of glucose intolerance and insulin resistance. (Adopted from: Kaur, 2014; Mendonça *et al.*, 2015).

Although obesity is not a prerequisite for IR diagnosis, studies have reported positive associations between the insulin-resistant state and abdominal obesity (Jennings *et al.*, 2009; de Boer *et al.*, 2011; Kaur, 2014). Additionally, several other factors, including geographical location of the study population, level of urbanisation, and the criteria used to diagnose IR or the MetS may influence the prevalence of IR (Erasmus *et al.*, 2012). Insulin resistance is associated with an adverse lipid profile, since studies have reported a positive association between TG levels and HOMA-IR, while HDL-c levels were inversely associated with the risk of IR in women (Jennings *et al.*, 2009; Sossa *et al.*, 2012; Delisle *et al.*, 2013). Here, IR indirectly elevate TG

levels by increasing the production and secretion of TG-rich very low density lipoprotein (VLDL) particles in the liver and decrease VLDL clearance due to the impaired activity of the lipoprotein lipase (LPL) enzyme (Sumner *et al.*, 2005; Ginsberg & Karmally, 2009).

Insulin and the insulin-like growth factor-1

Insulin share structural homology with insulin-like growth factor-1 (IGF-1), and interacts with the same membrane receptors with different affinities (Rajpathak *et al.*, 2012; Ren & Anversa, 2015). Insulin-like growth factor-1 is an anabolic hormone expressed in nearly all normal cells needed for growth and development, although its expression is dependent on age, gender, pubertal stage and occurrence of growth disorders, pituitary disease, and malnutrition (Braun *et al.*, 2011; Levine & Levine, 2014; Ren & Anversa, 2015). Synthesis of IGF-1 mainly occurs in the liver, and to a lesser degree in other tissues including muscle, cartilage, bone, nerves, and skin, where production is stimulated by growth hormone (GH) (Westley & May, 2013). The insulin-like growth factor binding proteins (IGFBPs) regulate IGF-1 activity (Ren & Anversa, 2015). Free IGF-1 (the ligand) binds to the IGF-1 receptor (IGF-1R) and activates the receptor, which in turn activates several other metabolic pathways, all of which are regulated by different IGFBPs that play a role in the pathogenesis of several diseases (Mendonça *et al.*, 2015; Ren & Anversa, 2015).

The insulin/IGF-1 pathway play a role in metabolic- and CVD, although the exact relationship between IGF-1 and metabolic diseases is still unclear (Ren & Anversa, 2015). Rajpathak *et al*. (2012) found free IGF-1 levels to be positively correlated with BMI and WC, while Friedrich *et al*. (2013) showed that IGF-1 levels were significantly higher in MetS participants. The proposed mechanism for this association is that obesity causes an elevation in the quantity of free IGF-1, due to its stimulatory effect on adipocyte differentiation (Rajpathak *et al*., 2012; Westley & May, 2013). Therefore, an increase in obesity results in an increase in free IGF-1 and a decrease in bound total IGF-1. Another proposed mechanism is that IGF-1 levels increase due to increased hepatic GH receptor expression, which stimulates hepatic IGF-1 synthesis (Rajpathak *et al*., 2012). Evidence suggests that IGF-1 affects glucose homeostasis *via* increased insulin levels, leading to decreased levels of IGFBPs affecting the bioavailability of IGF-1 and increasing free IGF-1 levels (Sierra-Johnson *et al*., 2008; Donohoe *et al*., 2012; Rajpathak *et al*., 2012).

On the other hand, Faupel-Badger *et al*. (2009) found that BMI and W:H were inversely related to IGF-1 levels in healthy women. This is possible, since obesity contributes to IR and hyperinsulinaemia, which may lead to GH receptor resistance, thereby decreasing IGF-1 levels (Westley & May, 2013). Low IGF-1 levels are associated with IR and T2DM, while individuals

with the MetS also presented with significantly lower IGF-1 levels compared to healthy controls (Friedrich *et al*., 2013; Ren & Anversa, 2015).

1.3.5 Dyslipidaemia

Dyslipidaemia is the most common cause of atherosclerotic CVD, and general characteristics of dyslipidaemia include increased TG, VLDL cholesterol (VLDL-c) and small LDL-c, and low HDL-c levels (summarised in Table 1.4 below) (IDF, 2006; Alberti *et al*., 2009; Jee & Jo, 2012). Increased VLDL-c and LDL-c levels also lead to increased apolipoprotein B (apo B) levels, which is directly associated with atherogenic dyslipidaemia (Jee & Jo, 2012).

Table 1.4: Cut-off values for dyslipidaemia.

	CUT-OFF VALUES	
	Men	**Women**
HDL-c	<1.03 mmol/L	<1.29 mmol/L
LDL-c	≥3.00 mmol/L	
TG	≥1.70 mmol/L	
Total cholesterol	≥5.00 mmol/L	

(Adopted from: IDF, 2006; Klug *et al*., 2015).

Several mechanisms exist whereby IR may cause atherogenic dyslipidaemia and CVD (Jee & Jo, 2012; Kaur, 2014). Under normal physiologic circumstances, insulin suppresses lipolysis, whereas impaired insulin signalling increases lipolysis, thus increasing the free fatty acid (FFA) concentration (Kirk & Klein, 2009; Kaur, 2014). These FFAs serve as substrate for the synthesis of TGs in the liver (Figure 1.6) (Kaur, 2014). Alternatively, the pathogenesis of dyslipidaemia causes an imbalance between the influx and production of FAs, which lead to a non-alcoholic fatty liver, and ultimately reduced HDL-c and elevated LDL-c levels (Kirk & Klein, 2009; Shapira, 2009; O'Neill & O'Driscoll, 2015).

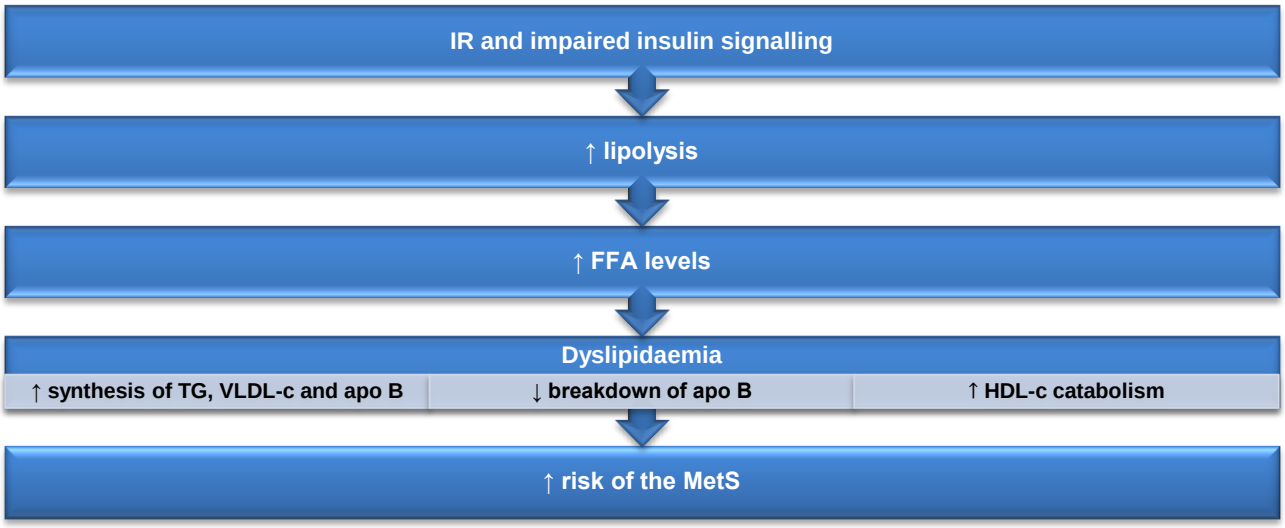

Figure 1.6: Pathophysiologic association between insulin resistance and dyslipidaemia.
(Adapted from: Kirk & Klein, 2009; Jeon *et al.*, 2011; Kaur, 2014; O'Neill & O'Driscoll, 2015).

From this section it is evident that obesity, and all other metabolic-related risk factors of the MetS, are all associated with each other, and also exhibit significant health implications. The specific link between adipose tissue and metabolic derangements *per se,* is thought to be partially facilitated by adipose tissue-derived adipokines (Shah *et al.*, 2012).

1.3.6 The role of adipokines

1.3.6.1 Introduction

Adipose tissue is both metabolically active and exerts an endocrine function (Doyle *et al.*, 2012). Factors such as adipose tissue mass and inflammation determine the synthesis and secretion of adipokines from VAT (Levine & Levine, 2014; Blüher & Mantzoros, 2015). Excess adipose tissue upregulate pro-inflammatory adipokine release, which causes an increased state of low-grade inflammation, while the anti-inflammatory mechanism is decreased (Donohoe *et al.*, 2011). Furthermore, changes in adipokine secretion is also associated with IR, and obesity-related pathologies that contribute to the MetS (Doyle *et al.*, 2012; Shah *et al.*, 2012; Abu-Farha *et al.*, 2014). Two of the most prominent adipokines, adiponectin and leptin, are found to be implicated in the MetS (Esteghamati *et al.*, 2011; Yun *et al.*, 2011; Cohen *et al.*, 2012; Shah *et al.*, 2012; Abu-Farha *et al.*, 2014).

1.3.6.2 Adiponectin

Adiponectin is almost entirely synthesised by WAT and plays a critical role in lipid- and glucose metabolism, enhancing hepatic and skeletal muscle insulin sensitivity, energy homeostasis, and act as an insulin-sensitising agent (Abu-Farha *et al.*, 2014). There also appear to be a negative

association between adiponectin and percentage body fat, WC and BMI, since a decreased secretion of adiponectin by the visceral-omental adipose tissue resulted in an inverse association between adiponectin and VAT (Shah *et al.*, 2012; Abu-Farha *et al.*, 2014). Comstock *et al.* (2014) found a significant inverse association between BMI and adiponectin levels. It is thus proposed that obesity can reduce adiponectin levels and contribute to IR in this manner, whereas higher adiponectin concentrations are associated with insulin sensitivity (Figure 1.7) (Braun *et al.*, 2011; Levine & Levine, 2014). Adiponectin levels can also either be positively or negatively associated with SBP, HOMA-IR, and HDL-c, TG, FBG levels in the context of the MetS (Shah *et al.*, 2012; Abu-Farha *et al.*, 2014; Atanassova *et al.*, 2014).

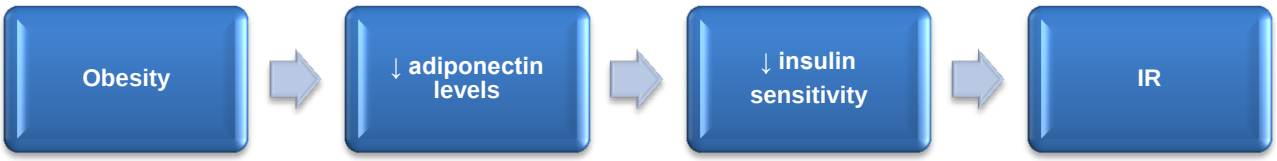

Figure 1.7: The effect of obesity on adiponectin levels that ultimately lead to insulin resistance.
(Adopted from: Braun *et al.*, 2011).

1.3.6.3 Leptin

Leptin is produced by WAT, epithelial cells of the stomach, bone marrow, pituitary, liver, and skeletal muscle. Furthermore, leptin functions to control eating behaviour, energy expenditure and balance, body weight, and plays a role in the immune and reproductive systems (Deng & Scherer, 2010; Braun *et al.*, 2011; Kaur, 2014).

Although several factors are known to affect leptin levels, not all studies are in agreement. Ganji *et al.* (2009) found no significant association between ethnicity and leptin levels, while a study by Cohen *et al.* (2012) reported leptin levels to be significantly higher in Black women. These ethnic differences in leptin levels may possibly reflect physiological and/or genetic differences in leptin production in adipose tissue (Cohen *et al.*, 2012). Obesity also influences leptin levels. Here, positive relationships were established between leptin levels and BMI, WC, total adipose tissue mass, percentage body fat, and regional adiposity (Yun *et al.*, 2011; Chiu *et al.*, 2012; Cohen *et al.*, 2012; Shah *et al.*, 2012; Nakamura *et al.*, 2013; Abu-Farha *et al.*, 2014; Motie *et al.*, 2014; de Castro *et al.*, 2015; García-Jiménez *et al.*, 2015). It was proposed that leptin contributes to body weight, and hence differences in leptin levels with increasing BMI are anticipated (Deng & Scherer, 2010; Kaur, 2014). Apart from increasing leptin levels in these states, a state of leptin resistance with concomitant impaired leptin- and insulin signalling follows, which diminishes the effects of leptin on appetite regulation, energy expenditure, and body weight (Figure 1.8) (Deng

& Scherer, 2010; Kaur, 2014; García-Jiménez *et al.*, 2015). Impaired leptin- and insulin signalling associated with the leptin-resistant state worsens the pro-oxidant and inflammatory state, further contributing to the development of IR, and obesity-related hypertension (García-Jiménez *et al.*, 2015).

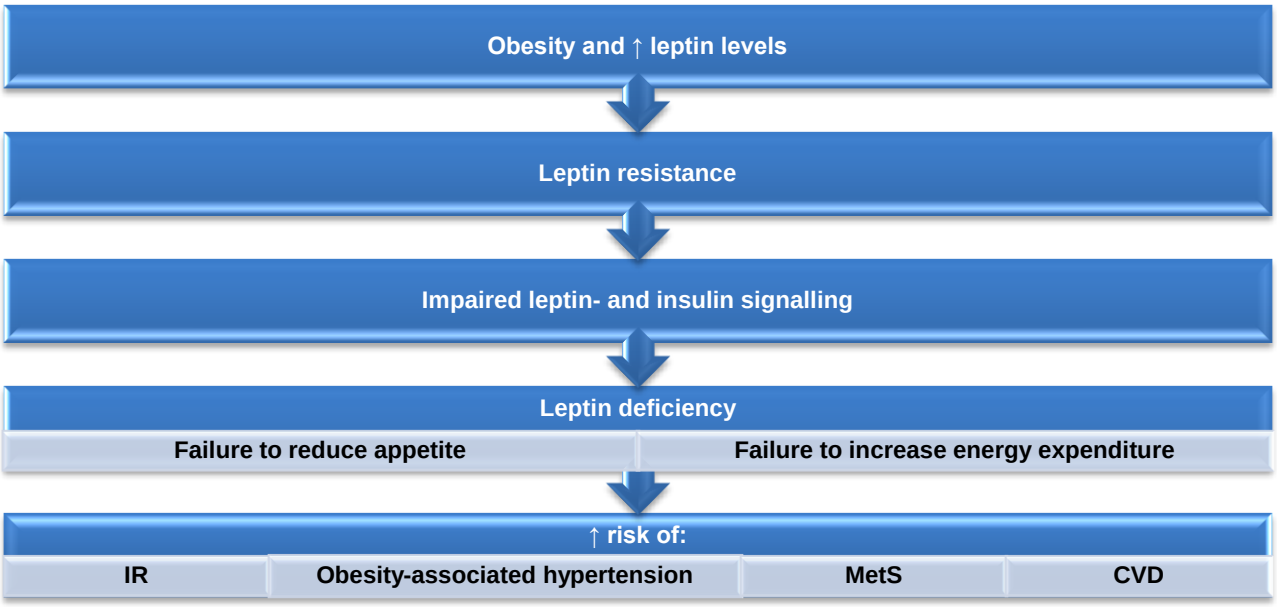

Figure 1.8: The relationship between obesity, leptin, and cardiovascular disease.
(Adapted from: Deng & Scherer, 2010; Kaur, 2014).

Leptin concentration is associated with various individual components of the MetS and are elevated specifically in people with the MetS (Esteghamati *et al.*, 2011; Chiu *et al.*, 2012; Shah *et al.*, 2012; Abu-Farha *et al.*, 2014; García-Jiménez *et al.*, 2015). Studies have also reported positive associations between hypertension and elevated leptin levels in women with and without the MetS (Yun *et al.*, 2011; Cohen *et al.*, 2012; Kaur, 2014). Elevated leptin levels were also found to be significantly associated with elevated HOMA-IR, FBG and insulin levels, and T2DM (Ganji *et al.*, 2009; Yun *et al.*, 2011; Chiu *et al.*, 2012; Cohen *et al.*, 2012; Shah *et al.*, 2012; Atanassova *et al.*, 2014; Abu-Farha *et al.*, 2014).

Elevations in leptin concentration are significantly associated with hypercholesterolaemia, hypertriglyceridaemia, elevated LDL-c, and reduced HDL-c levels (Yun *et al.*, 2011; Chiu *et al.*, 2012; Cohen *et al.*, 2012; Shah *et al.*, 2012). Leptin therefore plays an important role in facilitating energy homeostasis, and disruptions in leptin secretion may thus cause lipid changes, which could also result in FA profile alterations (Kawashima *et al.*, 2009). The FA profile is also affected by dietary intake and lipid metabolism, including lipolysis and *de novo* lipogenesis, which influences the physiological components related to the MetS (Hlavaty *et al.*, 2015).

1.4 FATTY ACIDS

1.4.1 Introduction

Fatty acids belong to a class of aliphatic monocarboxylic acids that have several functions, including; i) forming the basic building blocks of phospholipids and glycolipids found within the human body, ii) attachment to proteins to assist in directing these molecules to the correct place in the cell membrane, iii) storage as TGs, and iv) messenger molecules that act on the pancreatic β-cells to regulate glucose-stimulated insulin secretion (Horton *et al.*, 2006; Batchu *et al.*, 2009; Suburu *et al.*, 2013).

Humans acquire FAs by *de novo* lipogenesis from carbohydrates or proteins, or from dietary sources (Zong *et al.*, 2012; Suburu *et al.*, 2013). Regulation of FA metabolism is critical to maintain homeostasis, since changes in FA metabolism can lead to lifestyle-associated metabolic diseases (Zong *et al.*, 2012; Suburu *et al.*, 2013).

1.4.2 Basic physiology of fatty acids

The major biological role of fat is to store energy from carbohydrate-derived carbon precursors as compact FAs (Vessby *et al.*, 2002; Suburu *et al.*, 2013). *De novo* lipogenesis occurs in the FA synthesis pathway to produce saturated short-, and then medium chain FAs (Goodridge & Sul, 2009). Elongation and desaturation enzymes can further modify and esterify these FAs, and convert these to TG molecules to be stored (Vessby *et al.*, 2002; Goodridge & Sul, 2009; Zong *et al.*, 2012). *De novo* synthesised FAs can immediately be used as energy inside the cell, whereas dietary FAs require enzymes, transporters, and chaperone proteins to facilitate their absorption, transport, and uptake into cells (Suburu *et al.*, 2013).

Dietary FAs must be absorbed from the small intestine, transported as lipoprotein particles, and then enter the circulation *via* the lymphatic system (Suburu *et al.*, 2013). Once the lipoprotein particles bind to their respective receptors, the activity of the LPL enzyme releases the FAs (now FFAs), which is followed by cellular uptake (Horton *et al.*, 2006; Hodson *et al.*, 2008). Elongation and desaturation enzymes can modify these FFAs, similar to the FFAs synthesised by *de novo* lipogenesis (Suburu *et al*., 2013). Figure 1.9 illustrates the *de novo* lipogenesis of FAs and metabolism of dietary FAs. Fatty acid composition affects membrane properties, metabolic signalling, and energy expenditure, all of which are modulated by FA chain length and degree of saturation (Vessby *et al.*, 2002; Horton *et al.*, 2006). The desaturase enzymes catalyse desaturation of FAs, although these enzymes have different affinities for different FAs (Zong *et al.*, 2012). This desaturation process is dependent on the competition between substrates, and

product inhibition (Jackson *et al.*, 1997; Vessby *et al.*, 2002). Hence, the effectiveness of desaturation is not only dependent on the specific FAs, but the content of the other FAs competing for the desaturation enzyme also plays a role (Vessby *et al.*, 2002; Goodridge & Sul, 2009). The following section presents the different types of FAs.

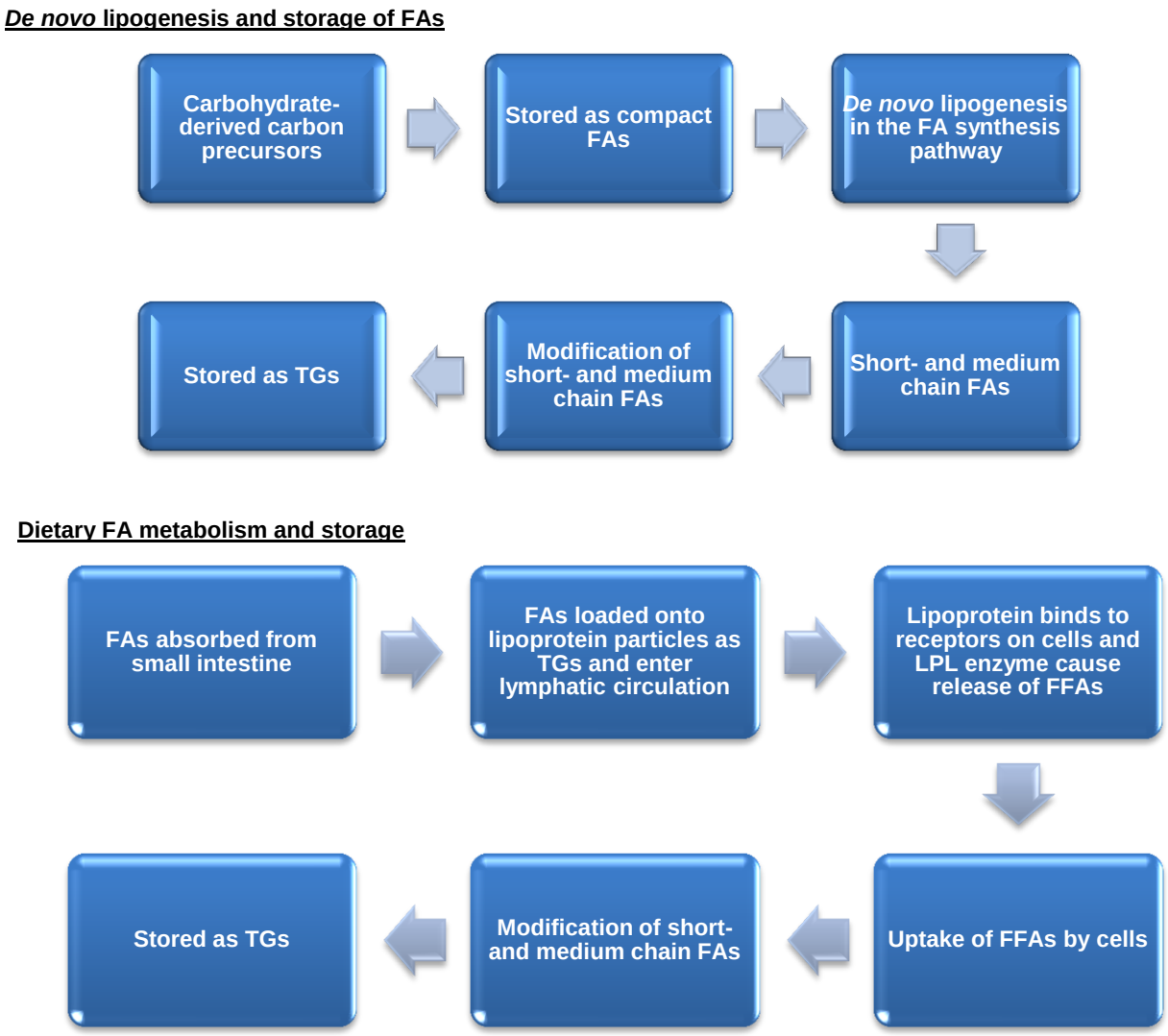

Figure 1.9: Basic fatty acid physiology illustrated for *de novo* lipogenesis and dietary fatty acids.
(Adapted from: Vessby *et al.*, 2002; Goodridge & Sul, 2009; Zong *et al.*, 2012; Suburu *et al.*, 2013).

1.4.3 Different types of fatty acids

The different FAs are functionally divided into short-, medium- and long chain FAs according to the number of carbons in the chemical structure (Bahrami, 2009). Saturated and unsaturated FAs constitute the two major FA types (Gillingham *et al.*, 2011; Hussein, 2013). Sources of unsaturated FAs include vegetable oils, whereas SFAs are more common in animal sources (Shapira, 2009). Saturated FAs contain hydrocarbons without double bonds, whereas unsaturated FAs have double bonds (Anderson & Ma, 2009). Unsaturated fats are subdivided

further into MUFAs and PUFAs, where the former has one double bond, and the latter at least two double bonds (Anderson & Ma, 2009; Hussein, 2013). Polyunsaturated FAs can further be subdivided into Ω-3 (also n-3), Ω-6 (also n-6), and Ω-9 (also n-9), depending on the position of the first double bond (Anderson & Ma, 2009; Gillingham *et al.*, 2011; Hussein, 2013). Figure 1.10 summarises the different FA classes:

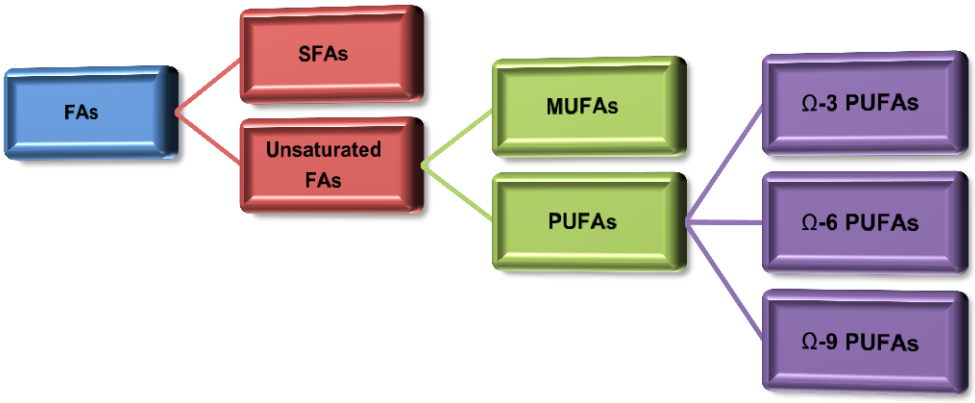

Figure 1.10: Classification of the different fatty acids.
(Adapted from: Anderson & Ma, 2009; Gillingham *et al.*, 2011; Hussein, 2013).

The two enzyme precursors that are needed to synthesise Ω-3 and Ω-6 FAs, elongase and desaturase, are not made in humans, which necessitates these essential FAs to be included in the diet (Spector, 2000; Hussein, 2013). Both the degree of saturation and the carbon chain length of the FA affect its biological function (Suburu *et al.*, 2013); however, several other factors may also influence FA levels. Accurate assessment of FA concentrations in different types of tissue, as well as in blood is imperative, since there is a clear relationship between FA, metabolic parameters, and the risk of NCDs (Harris, 2009).

1.4.4 Fatty acid assessment methods

1.4.4.1 Introduction

Most of the detection methods used to quantify FAs are done to also investigate relationships between other clinical and biochemical parameters. Other, indirect methods rely on dietary assessment methods, which, in conjunction with biochemical analysis, prove to be more useful than a single method (Hodson *et al.*, 2008; Patel *et al.*, 2010; Serra-Majem *et al.*, 2012; Hussein, 2013; Cottet *et al.*, 2015). The distribution of FAs varies greatly between the different tissues and this reflects metabolic changes and physiological functions (Baylin *et al.*, 2005).

1.4.4.2 Plasma fatty acids

Fatty acids are released from adipose tissue and transported in the plasma after complexing with albumin (Hodson *et al.*, 2008). The use of blood plasma to determine FA composition is a convenient and easy method; however, it only reflects lipid levels several days after a meal (Vessby *et al.*, 2002; Hodson *et al.*, 2008; Patel *et al.*, 2010; Serra-Majem *et al.*, 2012). Studies have shown that plasma FA composition corresponds with adipose tissue FAs, specifically for the PUFAs and MUFAs (Baylin *et al.*, 2005; Hodson *et al*., 2008), while others reported that plasma FA levels are more comparable to red blood cell (RBC) membrane FA composition (Sun *et al.*, 2007; Patel *et al*., 2010; Sertoglu *et al.*, 2014). Although plasma FAs are a potential surrogate marker of tissue FAs, sampling should occur in the post-absorptive state, since recent dietary intake affect plasma FA levels (Hodson *et al.*, 2008).

1.4.4.3 Red blood cell membrane fatty acids

Biological membranes consist of FAs, which play a role in the fluidity and integrity of the membrane, and activity of the membrane-associated enzymes (Spector, 2000; Suburu *et al.*, 2013; Zhang *et al.*, 2014). The FA composition of the RBC membrane is mainly regulated by endogenous FA synthesis, since these membranes lack *de novo* FA synthesis, and changes because of desaturation or elongation (Spector, 2000; Mahendran *et al.*, 2014). Various studies have shown that the RBC membrane FAs, which are unaffected by dietary fat intake, reflect medium-term (±120 days) tissue FAs, and are therefore regarded as a better measure than plasma FAs due to the longer lifespan of the RBCs (Jackson *et al.*, 1997; Patel *et al.*, 2010; Serra-Majem *et al.*, 2012).

The RBC membrane FAs are measured after complex lipid extraction, methylation, and separation, followed by quantification, or qualification with gas chromatography (GC) (Jackson *et al.*, 1997; Zhang *et al.*, 2012b). Although the assessment of FAs in RBC membranes is considered superior to that measured in plasma, the assessment of FAs in adipose tissue samples is generally considered the gold standard (Vessby *et al.*, 2002; Patel *et al.*, 2010).

1.4.4.4 Tissue fatty acids

Adipose tissue biopsies reflect FA levels of the preceding months; however, this method is not often used in epidemiological studies due to its invasiveness (Vessby *et al.*, 2002; Patel *et al.*, 2010). Subcutaneous adipose tissue is generally taken from the buttocks or abdomen, with small significant differences in the SAT FA content observed between these different sites, due to greater and lesser proportions of SFAs and MUFAs present in the abdomen respectively

(Byers & Gieseker, 1997; Hodson *et al.*, 2008). Although tissue FAs is representative of long-term (six to 18 months) FA levels, it is still unclear whether adipocyte size or changes in adipose function will affect FA levels in tissue (Hodson *et al.*, 2008). The FA profile is used to assess the risk of metabolic disease using individual FAs and ratios of specific FAs (Lemaitre *et al.*, 2009; Lv & Yang, 2012; Novgorodtseva *et al.*, 2013; Jurczyszyn *et al.*, 2014; Sertoglu *et al.*, 2014), which is the focus of the next section.

1.4.5 Fatty acid indices

There appears to be a linear relationship between FA concentrations in the blood, and FA metabolism, with some individual FAs implicated in certain disease states (Harris, 2009). Different FAs function relative to each other and homeostasis between these FAs is crucial for good health (Jackson *et al.*, 1997). Several FA indices have been identified as possible predictors for different diseases, including the Ω-3 index, Ω-3 to Ω-6 ratio (Ω-3:Ω-6), n-9 saturation index (SI), and the stearoyl-CoA desaturase (SCD) enzymes (Jackson *et al.*, 1997; Harris, 2009).

1.4.5.1 Omega-3 index

The Ω-3 index assesses the risk of CVD, dementia, and Alzheimer's disease, and is calculated as the percentage of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) of the total RBC membrane FAs (Lemaitre *et al.*, 2002; Harris, 2009; Witte *et al.*, 2014). An Ω-3 index of four percent or less is considered high risk for disease, while values greater than eight percent are indicative of a low disease risk (summarised in Table 1.5) (Harris, 2009; von Schacky, 2011).

Table 1.5. The risk of disease associated with the omega-3 index.

OMEGA-3 INDEX	RISK FOR DISEASE
≤4.00 %	High
4.10-8.00 %	Intermediate
≥8.10 %	Low

(Adopted from: Harris, 2009; von Schacky, 2011).

A study by Howe *et al.* (2014) found that the Ω-3 index is inversely associated with certain measures of adiposity, such as BMI and WC. A possible mechanism for the beneficial effects is that the Ω-3 PUFAs mediate insulin and carbohydrate control of lipogenic and glycolytic genes that promote fat oxidation and inhibit fat storage (Clarke *et al.*, 2001). Furthermore, the Ω-3 index is not affected by either the fed or the fasted state, although diet, age, BMI, gender, alcohol consumption, and Ω-6 FAs seem to play a role (von Schacky, 2011).

1.4.5.2 Omega-3 to omega-6 ratio

The Ω-3:Ω-6 is another clinically significant FA ratio which estimates the proportions of the Ω-3 FAs (EPA and DHA) to the Ω-6 FAs (linolenic acid (LA, 18:2n-6) and arachidonic acid (AA (20:4n-6)) (Odegaard, 2009). Functions of the Ω-6 FAs include its pro-inflammatory effects, the synthesis of lipid mediators, and its role in the production of cell membranes (Spector, 2000; Strandvik, 2011; Hussein, 2013; Suburu *et al.*, 2013). High Ω-6 levels may attenuate the beneficial effects of Ω-3 FAs, and for this reason the Ω-3:Ω-6 was established to determine chronic health implications (Shapira *et al.*, 2009). Clinically, the Ω-3:Ω-6 is considered important because the different FAs compete for the same elongase and desaturase enzymes in different metabolic pathways to affect cellular responses (Odegaard, 2009; Strandvik, 2011). For example, AA (Ω-6) competes with EPA and DHA (Ω-3) for enzymes and space in the cell membrane (Hussein, 2013).

Unlike Ω-3 and Ω-6 FAs, the Ω-9 FAs are not considered essential FAs because the human body can produce these FAs from unsaturated FAs. However, the Ω-9 FAs, in particular the n-9 SI and the SCD (Δ-9) enzymes play important roles in certain disease states (Jackson *et al.*, 1997; Kawashima *et al.*, 2009; Shannon *et al.*, 2010; Pouchieu *et al.*, 2014).

1.4.5.3 n-9 saturation index

The “saturation index” is a substrate-to-product ratio, and refers to the ratio of SFAs to MUFAs, (Chajès *et al.*, 2011). The n-9 SI is calculated as the ratio of stearic acid (SA, 18:0) to oleic acid (OA, 18:1n-9), and this is regarded as a measure of cancer risk, since cancer patients present with significantly lower values (Jackson *et al.*, 1997; Shannon *et al.*, 2010). An n-9 SI value between 0.7 and 1.0 is suggestive of cancer, whereas values below 0.7 are considered a marker of cancer (see Table 1.6) (Jackson *et al.*, 1997).

Table 1.6: The n-9 saturation index and associated cancer risk.

n-9 SI	CANCER RISK
0.70-1.00	Suggestive of cancer
<0.70	Marker of cancer

(Adopted from: Jackson *et al.*, 1997).

The concept behind this index is to compare the ratio of SA, the most common SFA, with OA, the most common MUFA (Chajès *et al.*, 2011). In cancer cases, SA levels are significantly lower, while OA levels are higher, possibly due to increased lipid peroxidation, and it is proposed that SA is directly involved in inhibition of tumour development (Chajès *et al.*, 1999; Pandey *et al.*, 2003).

1.4.5.4 Stearoyl-CoA desaturase enzymes

The SCD enzymes are responsible for the desaturation of FAs, *e.g.* palmitic acid (PA, 16:0) to palmitoleic acid (PLA, 16:1n-7), and SA (18:0) to OA (18:1n-9), which are produced as a result of *de novo* lipogenesis (Hodson *et al.*, 2008; Zong *et al.*, 2012). The Δ-9 SCD 1 and -2 enzymes mediate lipogenesis for the conversion of SFAs to MUFAs (Hodson *et al.*, 2008; Kawashima *et al.*, 2009; Patel *et al.*, 2010; Zong *et al.*, 2012; Kim *et al.*, 2013). The conversion of PA (a SFA) to PLA (a MUFA) to OA (a MUFA) occurs with the aid of the Δ-9 SCD 1 enzyme, whereas the Δ-9 SCD 2 enzyme catalyses the conversion of SA to OA (Vessby *et al.*, 2002; Goodridge & Sul, 2009). Figure 1.11 illustrates the effects of the desaturation enzymes.

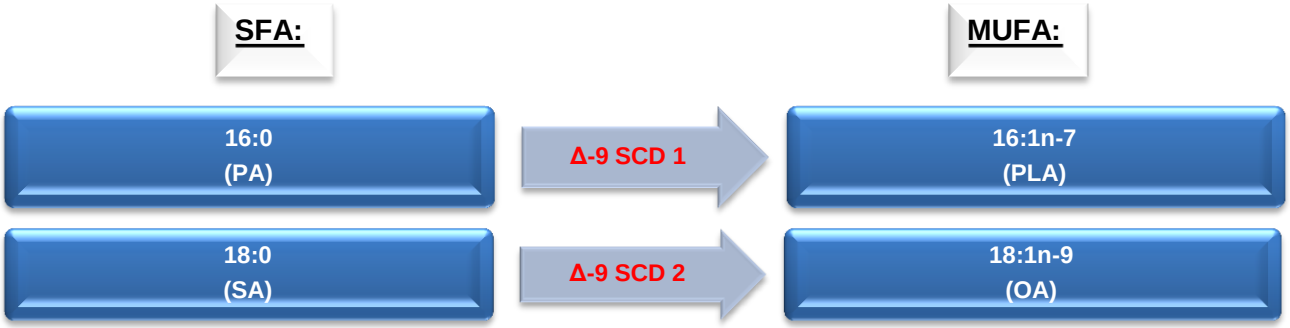

Figure 1.11: Conversion of saturated to monounsaturated fatty acids with the aid of the delta-9 SCD 1 and -2 and elongation enzymes.
(Adapted from: Vessby *et al.*, 2002; Goodridge & Sul, 2009).

Studies investigating desaturase activities generally report estimated desaturase activity values, rather than actual values, because direct measurement is a complex process (Kawashima *et al.*, 2009; Patel *et al.*, 2010). Therefore, indices of the different FAs are used as surrogate markers of enzyme activity (Patel *et al.*, 2010; Shannon *et al.*, 2010). It was shown that changes in SCD expression is related to concurrent changes in tissue FA levels, with these indices being calculated as the product-to-precursor ratio (Table 1.7) (Hodson *et al.*, 2008; Warensjö *et al.*, 2008).

Table 1.7: Calculation of the different desaturase enzymes using product-to-precursor ratios.

DESATURATION ENZYMES	PRECURSOR (SFA)	PRODUCT (MUFA)	RATIO (product ÷ precursor)
Δ-9 SCD 1	16:0	16:1n-7	(16:1n-7) ÷ (16:0)
Δ-9 SCD 2	18:0	18:1n-9	(18:1n-9) ÷ (18:0)

(Adapted from: Warensjö *et al.*, 2008; Patel *et al.*, 2010).

Changes in these indices have been associated with several metabolic alterations, including alterations in body fat, insulin sensitivity, the MetS, and cancer (Warensjö *et al.*, 2008; Kawashima *et al.*, 2009; Patel *et al.*, 2010; Pouchieu *et al.*, 2014). A possible explanation for this association is that SCD activity may influence FA metabolism by promoting MUFA synthesis and

decreasing FA oxidation (Chajès *et al.*, 2011). Monounsaturated FAs serve as mediators of cell differentiation, and ultimately an imbalance of these mediators can promote carcinogenesis (Chajès *et al.*, 2011). Lifestyle- (such as smoking and diet) and metabolic factors (obesity, BP, IR, and dyslipidaemia) may also have a negative impact on Δ-9 SCD activity.

1.4.6 Factors influencing fatty acid levels

1.4.6.1 Smoking status

Several studies reported on the negative impact of smoking status on FA levels – Hodson *et al.* (2008) reported significantly higher PLA and OA levels in smokers compared to non-smokers, and hypothesised that smoking alters the conversion of MUFAs to SFAs in blood lipids, thereby negatively affecting FA catabolism. Smokers have lower levels of Ω-3 PUFAs compared to non-smokers (Hodson *et al.,* 2008; Mozaffarian *et al.,* 2010; Chiuve *et al.*, 2012). Furthermore, smoking increases the conversion of the Ω-3 FA EPA to docosapentaenoic acid (DPA, 22:5n-3) (Hodson *et al.*, 2008). Harris *et al.* (2012) also suggested that the lower Ω-3 FA levels noted in smokers might be due to the oxidative destruction of the highly unsaturated FAs and/or alterations in Ω-3 metabolism.

1.4.6.2 Diet

Dietary intake is a major environmental determinant of the MetS (Grosso *et al.*, 2015). A Westernised diet is mostly associated with energy-dense processed products, snack foods, and fast and convenient meals (Feeley *et al.*, 2012; Steyn *et al.*, 2013; Bosu, 2014). Additionally, there is an increased consumption of red and processed meat, saturated and *trans*-fats, and decreased intake of fruit and vegetables, dietary fibre, and plant protein (Abrahams *et al.*, 2011; Steyn *et al.*, 2012; Dolman *et al.*, 2013; Shab-Bidar *et al.*, 2014). Studies have also reported significant positive associations between the consumption of different FAs and components of the MetS. For example, a high SFA intake is associated with obesity, reduced glucose metabolism and insulin sensitivity, IR, and elevated LDL-c levels (Anderson & Ma, 2009; de Oliveira *et al.*, 2012; Nettleton *et al.*, 2014; Shab-Bidar *et al.*, 2014).

Contrasting to the Westernised diet, the Mediterranean diet, rich in fruits and vegetables, whole grains and fish, and low in red meat and dairy products, which may reduce the risk of the MetS (Gotsis *et al.*, 2014; Grosso *et al.*, 2015). The health benefits associated with the Mediterranean diet depend on the high intake of antioxidants, PUFAs and MUFAs, and low intake of SFAs and *trans*-FAs (Grosso *et al.*, 2015). Poly- and monounsaturated FA consumption is associated with a reduced risk of developing the MetS, since it improves insulin sensitivity, and reduces BP and

LDL-c levels (Anderson & Ma, 2009; Nettleton *et al.*, 2014; Shab-Bidar *et al.*, 2014). High intake of SFAs upregulates desaturase activities thereby increasing MUFA levels, whereas PUFA intake is negatively associated with desaturase activities (Kawashima *et al.*, 2009; Bjermo & Risérus, 2010). Elevated *de novo* lipogenesis results in hypertriglyceridaemia, IR and obesity; a finding that is supported by significantly higher *de novo* lipogenesis in MetS participants compared to healthy controls (Zong *et al.*, 2013).

1.4.6.3 Metabolic syndrome risk factors

Changes in FA metabolism could possibly explain the link between visceral adiposity and metabolic dysfunction (Žák *et al.*, 2014). Studies have shown that the concentrations of some FAs differed significantly between individuals with the MetS and healthy controls. Here, SFA and MUFA levels were significantly higher, and PUFA levels significantly lower in MetS participants (Kabagambe *et al.,* 2008; Kawashima *et al.*, 2009; Novgorodtseva *et al.*, 2011; Zhang *et al.*, 2012a; Zong *et al.*, 2013; Žák *et al.*, 2014).

Since elevated levels of some FAs, including PA, PLA, and SA are associated with the number of MetS risk factors, it is anticipated that the different types of FAs will have diverse effects on the individual components of the MetS. For example, obesity contributes to changes in the FA profile, since overweight MetS participants presented with significantly higher SFA levels compared with normal weight counterparts (Klein-Platat *et al.*, 2005). Additionally, PLA levels were also positively associated with increased BMI and WC (Zong *et al.*, 2012). This notion is supported by the positive association between obesity and SCD activity, which is involved in the *de novo* synthesis of MUFAs from SFAs (Klein-Platat *et al.*, 2005; Warensjö *et al.*, 2005; Hlavaty *et al.*, 2015). Compared to normal weight persons, PUFA levels were significantly lower in overweight persons (Klein-Platat *et al.*, 2005). It is suggested that the Ω-3 FAs have beneficial effects on preventing and improving obesity by increasing FA β-oxidation, which reduces fat mass and body weight (Klein-Platat *et al.*, 2005; Kawashima *et al.*, 2009).

Studies have also reported that different FAs have opposing effects on BP, since BP was positively associated with elevated MUFA levels, and negatively with PUFA levels (Zhang *et al.*, 2012a; Zong *et al.*, 2012; Kim *et al.*, 2013). Kim *et al.* (2013) suggested then that the MetS-associated impaired FA metabolism, accelerates arterial stiffness, and this contributes to hypertension. Glucose intolerance and IR have also been shown to affect the FA profile, since OA and PLA levels were significantly associated with glycaemic status, while elevated Δ-9 SCD 1 activity was associated with IR (Bjermo & Risérus, 2010; Kurotani *et al.*, 2012; Cho *et al.*, 2014). This is possible since Δ-9 SCD activity is the rate-limiting enzyme for conversion of SFAs

to MUFAs, and this suggests that the Δ-9 SCD is protective against lipotoxicity caused by elevated SFA levels (Cho *et al.*, 2014). The association between Ω-3 FAs and insulin sensitivity remain unclear, since some found no clear association, whereas others found either inverse or positive associations (Sethom *et al.*, 2011; Kurotani *et al.*, 2012; Mirmiran *et al.*, 2012; Vanhala *et al.*, 2012; Mayneris-Perxachs *et al.*, 2014).

A positive relationship was also established between elevated cholesterol levels and PA and PLA levels, and Δ-9 SCD activity (Zong *et al.*, 2012; Hlavaty *et al.*, 2015). Additionally, hypertriglyceridaemia was thought to be the major link between FAs and the risk of the MetS, since *de novo* lipogenesis produce MUFAs, which are crucial substrates for TG synthesis (Zong *et al.*, 2013). In contrast to the SFAs and MUFAs, PUFA levels are associated with HDL-c levels, while a negative association was reported for TG levels (Mirmiran *et al.*, 2012; Vanhala *et al.*, 2012). This is possible since Ω-3 FAs have a beneficial effect on lipoprotein profiles through the promotion of FA β-oxidation, which reduces the synthesis of TGs in the liver (Kawashima *et al.*, 2012).

1.5 THE ASSOCIATION BETWEEN THE METABOLIC SYNDROME AND FATTY ACIDS

1.5.1 Introduction

The dysregulation of FA metabolism has been linked to several metabolic alterations (Kabagambe *et al.*, 2008; Patel *et al*., 2010; Novgordtseva *et al.*, 2011; Žák *et al*., 2014). When investigating individual SFA levels of MetS and non-MetS individuals, PA and SA levels were significantly higher in the former group (Nigam *et al.*, 2009; Tremblay-Franco *et al.*, 2015). Sethom *et al*. (2011) found similar results for PA levels in the MetS group; however, contrasting results were reported for SA levels. Neither Novgorodtseva *et al.* (2011), nor Žák *et al.* (2014) observed any significant difference in PA levels in the MetS and the non-MetS participants, although SA levels were significantly higher in the MetS groups. Reasons for these discrepancies may include the added effects of chronic diseases, since participants suffering from chronic diseases were included in the sample population, and these studies used different definitions of the MetS. The studies by Nigam *et al.* (2009) and Tremblay-Franco *et al.* (2015) used fasting plasma samples, adding to the possible contrasting results, since plasma FA levels reflect short-term FA intake (Patel *et al*., 2010; Serra-Majem *et al*., 2012). Although all of these studies used GC to determine FA levels, different methods were used to identify the individual FAs. For example, Sethom *et al*. (2011) used an internal standard, while studies by Novgorodtseva *et al.* (2011) and Tremblay-Franco *et al.* (2015) used different methylated FA

standards and controls for FA identification, and these different methods may explain the contrasting results.

Contrasting results have also been reported for both PLA and OA levels. Novgorodtseva *et al.* (2011) found PLA levels to be significantly lower in a MetS group, whilst others reported PLA levels to be significantly elevated (Nigam *et al.*, 2009; Sethom *et al.*, 2011; Žák *et al.*, 2014). The altered FA profile in MetS subjects might be related to both metabolic- and dietary changes, since Nigam *et al*. (2009) reported a positive association between PA levels and IR, suggesting that impaired insulin sensitivity was associated with increased Δ-9 SCD 1 activity. Mayneris-Perxachs *et al*. (2014) also noted significant positive associations between PA levels and the individual components of the MetS, including WC, TG and glucose levels, and suggested that individuals with the MetS have significantly higher SFA levels, that triggers higher Δ-9 SCD 1 activity that may lead to significantly higher PLA levels. Furthermore, lifestyle factors such as physical inactivity and dietary intake that promote obesity, may affect Δ-9 SCD 1 enzyme activity, since obesity is associated with reduced FA oxidation (Warensjö *et al.*, 2005; Sethom *et al.*, 2011).

Studies by Nigam *et al.* (2009) and Žák *et al.* (2014) found that OA levels did not differ; however, Sethom *et al.* (2011) found that OA levels were significantly higher in the MetS group. Glucose is an activator of the Δ-9 SCD 2 enzyme that is needed for the conversion of SA to OA (Vessby *et al.*, 2002). A significant difference in OA levels can be explained by the higher FBG levels; therefore, increased Δ-9 SCD 2 enzyme activity, and increased OA levels in the MetS group in the study by Sethom *et al.* (2011). Furthermore, the study by Nigam *et al.* (2009) included participants with chronic diseases and medications, which could also play a role in the contrasting findings.

Sethom *et al*. (2011) reported significantly higher Δ-9 SCD 2 activity in participants with the MetS, but a more recent study by Mayneris-Perxachs *et al*. (2014) found that the Δ-9 SCD 2 was significantly lower. Contrasting to others, Žák *et al.* (2014) reported no significant difference in Δ-9 SCD 2 activity of MetS and non-MetS participants. These studies however differed on several levels, including study design, inclusion and exclusion criteria, as well as the use of different MetS definitions.

Elevated total PUFA levels were significantly associated with a reduced risk of the MetS (Mayneris-Perxachs *et al*., 2014). Although some studies did not report any significant differences between α-linolenic acid (α-LA, 18:3n-3) or DPA levels of persons with and without the MetS (Nigam *et al.*, 2009; Sethom *et al.*, 2011; Žák *et al.*, 2014), Cespedes *et al*. (2015)

found that α-LA levels in individuals with the MetS were significantly lower. Also, even though both the studies by Nigam *et al.* (2009) and Cespedes *et al*. (2015) used the ATP III to diagnose the MetS, contrasting results were obtained, while Sethom *et al.* (2011) and Žák *et al.* (2014) obtained similar results using the IDF definition. Interestingly, the study by Sethom *et al*. (2011) used plasma samples, whereas Cespedes *et al.* (2015) used SAT biopsies with similar outcomes. The SAT represents the long-term integrated measure of exposure to FA dietary intake, and desaturation and elongation (Cespedes *et al.*, 2015).

Omega-3 FA levels also differed between the MetS and non-MetS groups. Nigam *et al.* (2009) and Zhang *et al*. (2012a) found that EPA and DHA levels of individuals with the MetS were significantly lower compared to healthy controls. Although Sethom *et al.* (2011) did not find any significant differences for EPA levels; DHA levels were significantly lower as compared to the results of Nigam *et al.* (2009). Here, the different findings between these two studies may be ascribed to the inclusion of individuals with history of acute myocardial infarction. Differences in the Ω-3 FA levels of persons with the MetS consequentially led to a significant difference in the Ω-3 index between the MetS and non-MetS groups, where the MetS group presented with a significantly lower Ω-3 index compared to controls (Nigam *et al.*, 2009).

Referring to the Ω-6 FAs, no significant differences were noted between the AA levels of persons with and without the MetS (Nigam *et al.*, 2009; Žák *et al.*, 2014); however, studies by Sethom *et al.* (2011) and Novgorodtseva *et al.* (2011) both reported that AA levels were significantly lower. Omega-3 and Ω-6 FA levels were lower and higher respectively, and this resulted in a significantly lower Ω-3:Ω-6 in the MetS compared to the non-MetS group (Nigam *et al.*, 2009; Sethom *et al.*, 2011). Even though differences in FA levels were observed between the MetS and non-MetS groups, it is not known whether one or more of the MetS risk factors are responsible for these differences. The individual components of the MetS and its effects on FA levels will be discussed in the following sections.

1.5.2 Metabolic syndrome risk factor effects on fatty acids

1.5.2.1 Obesity

Fatty acids are considered major physiological role players in obesity and IR (Boden, 2009). Adipose tissue stores excess calories as TG to maintain glucose- and lipid homeostasis (Tuvdendorj *et al.*, 2013). Obesity stimulates lipolysis resulting in mobilising FFAs into circulation (Boden, 2009; Mendonça *et al*., 2015). The elevated FFAs lead to an increase in gluconeogenesis, TGs, and VLDL-c (illustrated in Figure 1.12) (Boden, 2009; Mendonça *et al*.,

2015). Additionally, the FFAs affect insulin sensitivity that inhibit glucose uptake and result in increased insulin secretion, causing a state of hyperinsulinaemia that ultimately leads to IR (Boden, 2009; Mendonça *et al*., 2015).

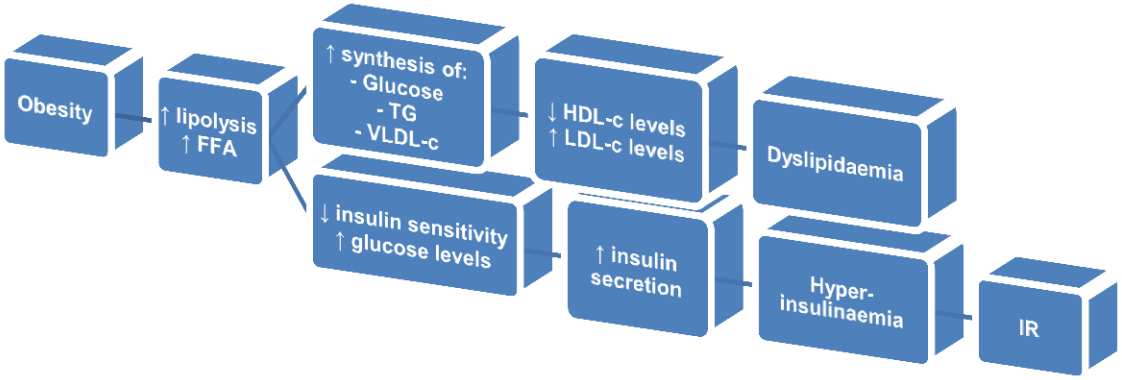

Figure 1.12: Health implications of obesity on dyslipidaemia and hyperinsulinaemia.
(Adapted from: Boden, 2009; Mendonça *et al*., 2015).

There are distinct differences in the FA composition of normal weight, overweight, and obese individuals with and without the MetS. It was reported that FA levels in obese persons were significantly higher than those of normal weight individuals (Klein-Platat *et al.*, 2005; Jeon *et al*., 2011). Studies by Klein-Platat *et al.* (2005) and Tremblay-Franco *et al.* (2015) specifically investigated these differences and found that MetS obese people presented with higher levels of SA and PA compared to non-MetS obese and normal weight participants. Although no significant differences were reported for OA levels, the results also pointed to distinct differences in the activity of the Δ-9 SCD 1 and -2 enzymes between these groups, since the activity of the Δ-9 SCD 1 enzyme increased, while that of the Δ-9 SCD 2 decreased (Tremblay-Franco *et al.*, 2015). A positive association was also reported between Δ-9 SCD activity and WC, which is suggestive of reduced FA oxidation and increased *de novo* lipogenesis, which may be related to the development of non-alcoholic fatty liver disease (Kawashima *et al*., 2009; Vinknes *et al.*, 2013; Tremblay-Franco *et al.*, 2015). Visceral obesity is associated with increased lipolytic activity, increased hepatic FA uptake, and reduced FA oxidation, which lead to hepatosteatosis (Golbidi *et al.*, 2012). Additionally, elevated SFA levels also induce hepatosteatosis, which is also directly associated with IR and non-alcoholic fatty liver disease (Golbidi *et al.*, 2012; Vessby *et al.*, 2012).

This is in contrast to the beneficial effects of the Ω-3 FAs on obesity, since these FAs increase FA oxidation to lower body weight (Kawashima *et al.*, 2009). However, DPA, DHA, and EPA levels did not differ between normal weight and obese persons with or without the MetS, while AA levels were significantly lower in normal weight compared to obese persons without the

MetS (Tremblay-Franco *et al.*, 2015). Different FAs have been associated with measures of obesity, since Kabagambe *et al.* (2008) found that BMI and WC were positively correlated with SFA and Ω-6 levels, and negatively correlated with MUFA and Ω-3 levels, as well as the Ω-3 index in participants with the MetS. Polyunsaturated FAs have been shown to contribute to the regulation of lipogenesis and fat oxidation, and can contribute to obesity and the MetS in this manner (Kabagambe *et al.*, 2008).

Additionally, a reduced capacity of adipose tissue to store TGs can lead to a flux of FFAs to other tissues, which promote the redistribution of fat (Tuvdendorj *et al.*, 2013). Increasing subcutaneous adipocyte size is associated with adipocyte IR and hypertrophy, which cause increased lipolysis, and reduced TG storage in SAT (Tuvdendorj *et al.*, 2013; Heemskerk *et al.*, 2015). Upper body SAT accounts for the majority of FFAs that are released into systemic circulation, despite VAT expansion, a mechanism which is referred to as the "FA theory" (Grundy, 2015). The quantity and the type of FAs released play an important role in the pathophysiology of obesity (Heemskerk *et al.*, 2015). Subcutaneous adipose tissue of obese women contained significantly higher levels of EPA, DPA, and DHA compared to controls, possibly to reduce obesity-associated inflammation, since Ω-3 FAs have anti-inflammatory properties (Heemskerk *et al.*, 2015).

Fatty acids have also been shown to interfere with BP, since both the Ω-3 PUFAs, EPA and DHA, are known precursors of lipoxins, resolvins and protectins, which are all involved in the regulation of vascular tone and BP (Kabagambe *et al.*, 2008; Novgordtseva *et al.*, 2011; Sethom *et al.*, 2011).

1.5.2.2 Hypertension

Different studies have shown that PUFA levels were negatively correlated with SBP and DBP, whereas Ω-3 FAs were shown to exert beneficial effects on BP *per se* (Kabagambe *et al.*, 2008; Sethom *et al.*, 2011; Cabo *et al*., 2012). Following desaturation and elongation, α-LA is converted to EPA, which is a precursor of three series of prostaglandins and five series of leukotrienes, which have been shown to play a role in mediating vasoconstriction (Spector, 2000; Cabo *et al*., 2012). Next, EPA is elongated and desaturated to DHA (Cabo *et al*., 2012). Figure 1.13 illustrates the metabolism of Ω-3 and -6 PUFAs, and its relation to BP.

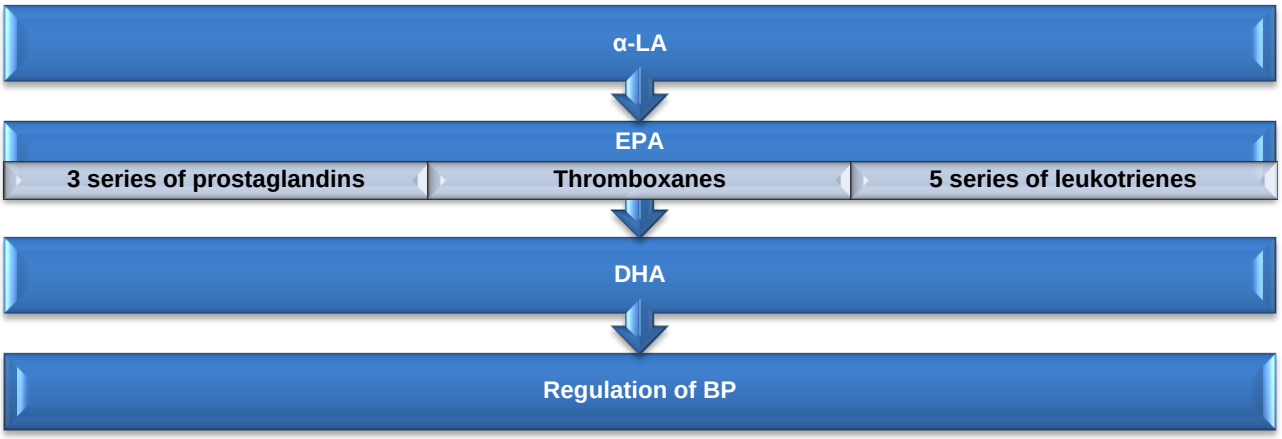

Figure 1.13: Metabolism of omega-3 fatty acids in relation to blood pressure.
(Adapted from: Novgordtseva *et al.*, 2011; Cabo *et al*., 2012).

A study by Zhang *et al.* (2012a) found that participants in the highest DHA quartile had a significantly lower risk of developing hypertension, whereas a very recent study did not find this relationship (Cespedes *et al.*, 2015). These studies had different study designs, since the former study was a case-control, while the more recent study by Cespedes *et al.* (2015) was a cross-sectional study. Different methods to study the FA profiles were also used and this may have influenced the findings, since the RBC membrane FAs reflect medium-term FA exposure, while gluteal SAT reflects long-term FA exposure.

Blood pressure may further be influenced by the SFAs, since studies by Kabagambe *et al.* (2008) and Novgordtseva *et al.* (2011) found significant positive associations between SBP and SFA levels of MetS participants. Results from a study by Novgordtseva *et al.* (2011) indicated that the MetS could lead to various changes in FA composition: (i) it can affect the charge on cell membranes, (ii) it can increase membrane microviscosity, (iii) it can activate a pro-inflammatory state, and (iv) it can increase the sensitivity of arterial smooth muscle cells to result in vasoconstriction effects. This chain of events contributes to hypertension (Figure 1.14).

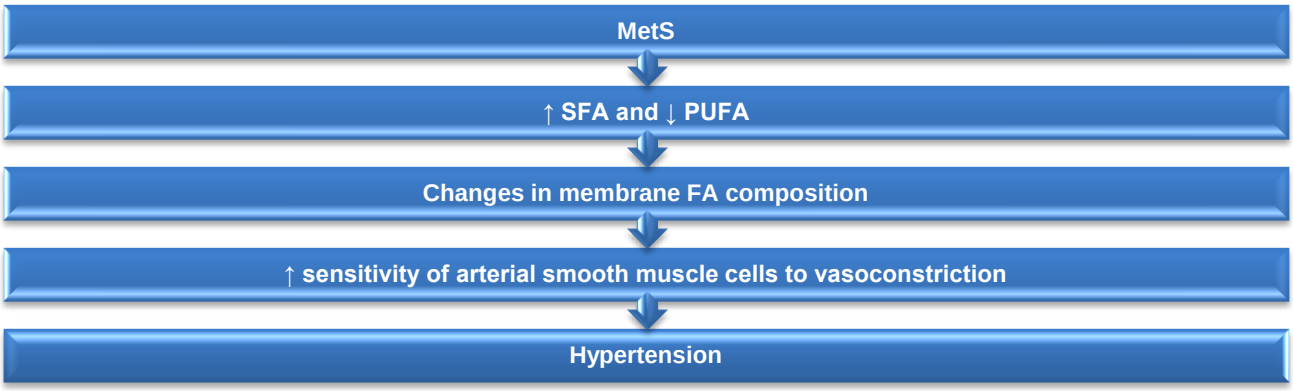

Figure 1.14: Effect of metabolic syndrome and the associated changes in fatty acid composition on hypertension.
(Adopted from: Novgordtseva *et al.*, 2011).

Not only do FAs affect BP, but insulin sensitivity is also affected, since several studies indicated that MUFA levels are positively associated with insulin levels, and IR (Nigam *et al.*, 2009; Sethom *et al.*, 2011; Kurotani *et al.*, 2012; Sertoglu *et al.*, 2014). In agreement, Cho *et al.* (2014) also reported significantly higher PLA levels in MetS compared with non-MetS participants. This is likely, since insulin stimulates hepatic *de novo* lipogenesis, and reduces FA oxidation (Sertoglu *et al.*, 2014).

1.5.2.3 Glucose intolerance and insulin resistance

The occurrence of IR causes adipose tissue hypertrophy, which then releases large quantities of FFAs into the hepatic portal vein. These FFAs are transported to the liver where it is believed to upregulate glucose release, as well as TG- and VLDL particle synthesis (Levine & Levine, 2014; Mendonça *et al*., 2015). This causes a state of lipotoxicity that impair glucose-stimulated insulin release, and ultimately leads to β-cell apoptosis and dysfunction (Levine & Levine, 2014). Generally, the shift from impaired glucose tolerance to T2DM is characterised by elevated plasma FFAs (Mendonça *et al*., 2015). Both IR and T2DM are related to lipid disorders, including FFA disorders, since elevated FFAs released from adipocytes damage pancreatic β-cells, and affect insulin signalling pathways (Liu *et al*., 2010a).

Positive correlations were evident between SFA levels and glucose- and insulin levels, as well as for the HOMA-IR in participants with the MetS (Kabagambe *et al.*, 2008; Patel *et al*., 2010). A 2011 study that compared individuals with the MetS, and individuals with the MetS and IR to healthy controls, found that both experimental study groups presented with significantly higher SFA levels (Novgorodtseva *et al*., 2011). Glucose, insulin, and the HOMA-IR were also positively associated with MUFA levels (Sethom *et al.*, 2011; Mayneris-Perxachs *et al*., 2014). The MetS is associated with elevated FBG and insulin levels, which enhance hepatic *de novo* lipogenesis and reduce FA oxidation (Sethom *et al.*, 2011). Activity of the Δ-9 SCD 1 enzyme was significantly associated with reduced insulin sensitivity, and the MetS (Kröger *et al*., 2010; Mahendran *et al*., 2014; Mayneris-Perxachs *et al*., 2014). This is possible since elevated PA levels in the insulin-resistant state prevent glucose uptake in insulin-sensitive cells, and elevated glucose levels activate the Δ-9 SCD 1 enzyme to convert PA to PLA, resulting in elevated PLA levels (Vessby *et al*., 2002).

Endogenous SFA synthesis is increased due to increased glucose- and insulin uptake, since glucose and insulin are activators of the Δ-9 SCD 2 enzyme that converts SA to OA (Figure 1.15) (Vessby *et al*., 2002; Kawashima *et al*., 2009; Mayneris-Perxachs *et al.,* 2014; Sertoglu *et al*., 2014). However, T2DM patients present with low insulin levels, which are therefore

insufficient to activate the Δ-9 SCD 2 enzyme, which is vital for the conversion of SA to OA (Sertoglu *et al*., 2014). Hence, individuals with T2DM presents with significantly higher SA levels compared to controls. However, the same study also found that RBC membrane OA levels were significantly higher in the T2DM group compared to controls, which reflect increased lipogenesis and Δ-9 SCD 2 enzyme activity (Igal, 2011; Sertoglu *et al*., 2014). These associations may be due to the influence of FA composition on cell membrane functions, because changes in SFA levels affect membrane fluidity, ion permeability, and insulin receptor binding (Patel *et al*., 2010).

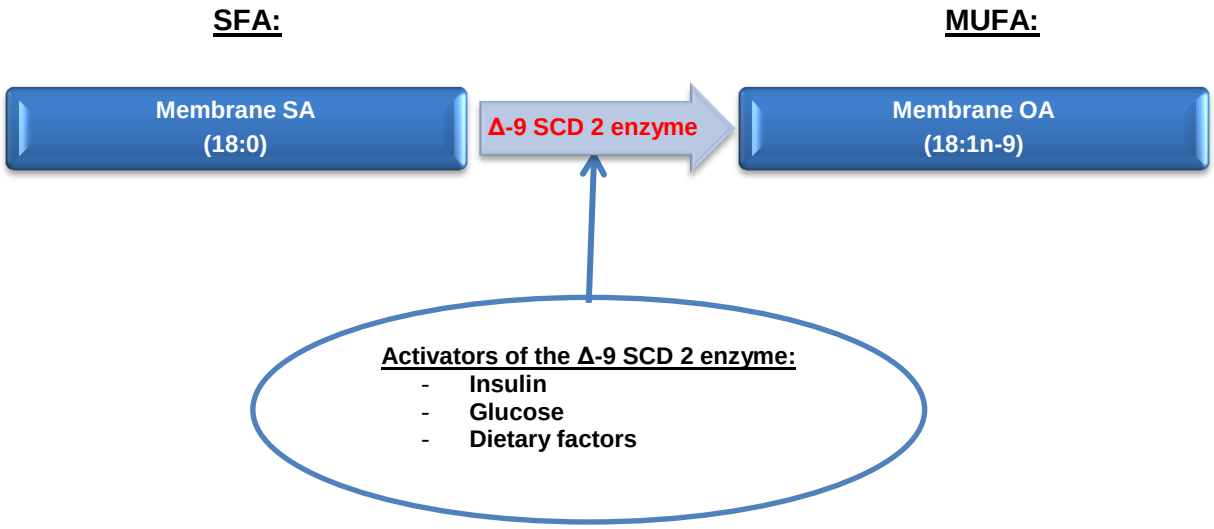

Figure 1.15: Major activators of the delta-9 SCD 2 enzyme, that is necessary for conversion of stearic to oleic acid in humans.
(Adapted from: Vessby *et al*., 2002; Sertoglu *et al*., 2014).

In contrast to the adverse effects of SFA on insulin sensitivity, Nigam *et al*. (2009) noted an inverse association between the Ω-3 FAs and IR in MetS participants. It was hypothesised that this inverse association is related to the beneficial effects of the Ω-3 FAs on insulin signalling (Clarke *et al.*, 2001; Kabagambe *et al.*, 2008; Nigam *et al*., 2009). In agreement, a 2011 study comparing individuals with the MetS, and individuals with the MetS and IR to healthy controls, found that both experimental study groups presented with significantly lower PUFA levels, (Novgorodtseva *et al*., 2011).

Kabagambe *et al.* (2008) reported significant negative associations between insulin and the HOMA-IR, and the Ω-6 FAs, thereby suggesting that Ω-6 FAs may have beneficial effects on IR. Furthermore, Sartorelli *et al.* (2010) also noted an increased Ω-3:Ω-6, which was associated with normal glycaemic status in persons with IR. Linolenic acid was also shown to have beneficial effects on glucose metabolism in these insulin-resistant persons, independent of dietary SFA intake, hypertension, hypertriglyceridaemia, and abdominal obesity. Sartorelli *et al.* (2010) then suggested that it is likely that an inflammatory process is involved in the association between LA and IR.

Dietary intake of different fat subtypes may affect the risk of IR and glucose intolerance by altering the phospholipid composition of cell membranes, and this may affect insulin secretion that ultimately cause dysregulation of blood glucose levels (Sartorelli *et al*., 2010). Fatty acids have also been shown to interfere with the lipid profile, since elevated TG levels are positively correlated with MUFA levels, especially PLA and OA levels, in participants with the MetS (Zong *et al*., 2012, 2013; Mayneris-Perxachs *et al*., 2014; Hlavaty *et al*., 2015). Mozaffarian *et al*. (2010) also reported that PLA was associated with favourable HDL-c levels.

1.5.2.4 Dyslipidaemia

The positive association reported between MUFAs and TG levels is probable, since PLA is a direct regulator of metabolism, it is a primary product of the Δ-9 SCD 1, and is a crucial component for the synthesis of TGs (Mozaffarian *et al*., 2010; Zong *et al*., 2012). Vinknes *et al*. (2013) reported a positive association between cholesterol levels and Δ-9 SCD 1 activity, while Mayneris-Perxachs *et al*. (2014) also reported a positive association between Δ-9 SCD 1 and -2, and TG levels. A significant negative association was also noted for Δ-9 SCD 2 and HDL-c levels (Mayneris-Perxachs *et al*., 2014).

The SFAs have been implicated in dyslipidaemia, altering both lipoprotein metabolism and -concentrations (Ruiz-Núñez *et al*., 2016). However, the association between SFAs and lipid levels vary according to the specific SFA that is studied, since some studies found that PA have unfavourable effects on the lipid profile, while other SFAs such as SA were associated with a favourable lipid profile (Mayneris-Perxachs *et al*., 2014; Hlavaty *et al*., 2015). This was also evident in an earlier study by Zong *et al*. (2013) that reported a negative association between SA and TG levels. It is proposed that differences in chain length might be responsible for these contrasting effects of the different SFAs on the lipid profile (Micha & Mozaffarian, 2010).

An earlier study by Klein-Platat *et al*. (2005) reported a weak negative association between the Ω-3 FAs and TG levels, while a positive association was noted for HDL-c levels. In contrast, Kabagambe *et al*. (2008) and Nigam *et al*. (2009) found no significant relationship between the Ω-3 FAs and any of the blood lipid fractions. Studies that are more recent also noted inverse associations between TG levels and individual Ω-3 FAs, and the Ω-3 index, which is possible since Ω-3 FAs reduce β-oxidation and hepatic production of TGs (Shearer *et al.*, 2011; Harris *et al*., 2012; Mirmiran *et al*., 2012). The differences between these studies may be attributed to the inclusion of different Ω-3 FAs, since the effects of the individual Ω-3 FAs differ. For example, α-LA exerts most of its effects by modulating lipoproteins, whereas EPA and DHA reduce TG synthesis and adiposity (Poudyal *et al*., 2011). Kabagambe *et al*. (2008) reported significant

negative associations between TG and LDL-c levels, and the Ω-6 FAs, which is possible since IR and the MetS are associated with reduced TG clearance. Mayneris-Perxachs *et al.* (2014) supported this finding, by reporting an inverse relationship between LA and TG levels, and a greater LA deficiency, which was associated with greater TG biosynthesis.

Although various studies have attempted to determine the negative effects of the MetS, and its risk factors on FA levels, long-term FA intake may actually be of benefit. The next section will explore the effect of long-term FA intake on the MetS status and the components thereof.

1.5.3 Effect of long-term fatty acid intake on metabolic syndrome status and its risk factors

Long-term studies have proposed that eating a balanced healthy diet, especially focussing on PUFA intake; can reduce the risk of the MetS and/or its components (Buckley & Howe, 2009; Serra-Majem *et al*., 2012; Braeckman *et al*., 2013).

Few, and inconsistent results exist for the effects of long-term FA supplementation on the risk of the MetS. The consumption of Ω-3 PUFAs (found in fatty fish, seafood, and lean meat) has shown several health benefits, such as reducing BP and markers of systemic inflammation, improving IR and the lipid profile, and reducing the risk of the MetS (Buckley & Howe, 2009; Cabo *et al.*, 2012; Harris *et al.*, 2012; Serra-Majem *et al*., 2012). However, Zhang *et al*. (2012a) did not find any association between long-term EPA and DPA intake and the risk of the MetS. Specifically in the context of obesity, dietary intake of Ω-3 FAs was associated with modest reductions in body weight, and improvement in body composition in people with the MetS (Buckley & Howe, 2009; Tierney *et al.*, 2011). The proposed mechanism here was that enhancement of postprandial satiety led to lower dietary intake, enhanced fat oxidation, and regulation of adipogenesis to reduce fat deposition (Buckley & Howe, 2009). Although Ω-3 supplementation reduced body weight, WC measurements remained unchanged in this group (Tierney *et al.*, 2011). Compared to diets supplemented with Ω-3 or -6 FAs, MUFA supplementation also seemed to reduce SBP in persons with the MetS, as well as in healthy controls (Gillingham *et al.*, 2011; Tierney *et al.*, 2011; Grieger *et al.*, 2014).

Monounsaturated FAs have been shown to regulate glycaemic response and improve insulin sensitivity. Nonetheless, dietary interventions to reduce SFA intake did not show any improvement in insulin sensitivity, fasting insulin and FBG levels or the HOMA-IR in people with the MetS (Gillingham *et al.*, 2011; Tierney *et al.*, 2011). Similarly, a study by Clevenger *et al.*

(2015), comparing the effects of diets supplemented with different fat types (SFA, MUFA and PUFA), did not find significant changes in FBG levels.

Following SFA and MUFA supplementation, HDL-c levels in persons with the MetS improved (Gillingham *et al.*, 2011; Tierney *et al.*, 2011). Increased MUFA intake was shown to reduce TG levels in obese and overweight participants with the MetS (Gillingham *et al.*, 2011). A Ω-3 supplementation study by Grieger *et al.* (2014) did not find any significant differences in blood lipid levels. From the studies mentioned above, evidence seems to suggest that MUFA supplementation may be more beneficial in altering the different risk factors of the MetS compared to PUFA supplementation (Gillingham *et al.*, 2011). These effects are summarised in Figure 1.16.

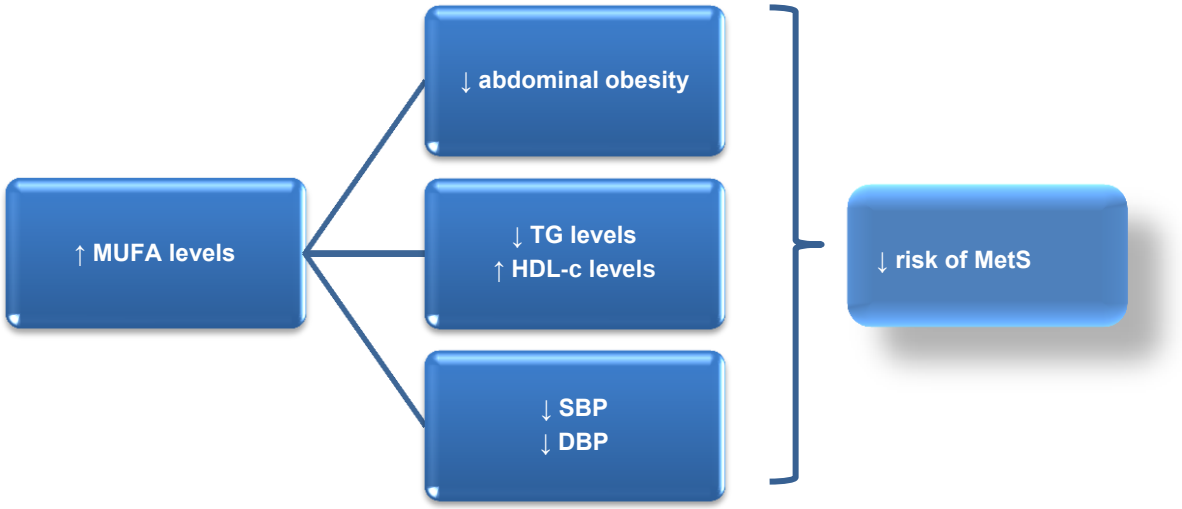

Figure 1.16: The beneficial health effects of dietary monounsaturated fatty acid intake.
(Adapted from: Gillingham *et al.*, 2011; Tierney *et al.*, 2011; Grieger *et al.*, 2014).

1.6 ASSOCIATION BETWEEN THE METABOLIC SYNDROME AND CANCER

1.6.1 Introduction

Scientific evidence describes the relationship between the MetS and increased risk of certain lifestyle-associated cancers (Kuchiba *et al.,* 2012; Leiba *et al*., 2012; Reeves *et al*., 2012; Kawai *et al*., 2013; Wiedman *et al*., 2013; Li *et al*., 2014; Lindkvist *et al*., 2014; Roswall *et al*., 2014; Almquist *et al*., 2015). Although the exact mechanisms remain unclear, it has been claimed that obesity might be implicated. This is largely due to evidence showing that increased adiposity results in a pro-tumorigenic state, since adipose tissue hypertrophy causes the infiltration of macrophages and T-cells that produce adipokines (Donohoe *et al.*, 2010; Blüher & Mantzoros, 2015). Additionally, adiposity is associated with hyperinsulinaemia and elevated IGF-1 levels, contributing to the promotion of cancer development (Donohoe *et al*., 2010).

1.6.2 Metabolic syndrome risk factors, biochemical risk factors and cancer

Many studies have shown that the components of the MetS are major risk factors for several cancer types (Häggström *et al*., 2011; Kuchiba *et al*., 2012; Leiba *et al*., 2012; Kawai *et al*.,2013; Wiedman *et al*., 2013; Li *et al*., 2014; Lindkvist *et al*., 2014; Roswall *et al*., 2014). Factors associated with the MetS, such as obesity, and elevated FBG and insulin levels have all been associated with an increased cancer risk (Johansen *et al.*, 2010; Wulaningsih *et al.*, 2012; Lindkvist *et al.*, 2013; Westley & May, 2013; Wiedman *et al*., 2013; Li *et al*., 2014). Figure 1.17 illustrates the increasing risk for cancer with increasing BMI, and FBG and insulin levels.

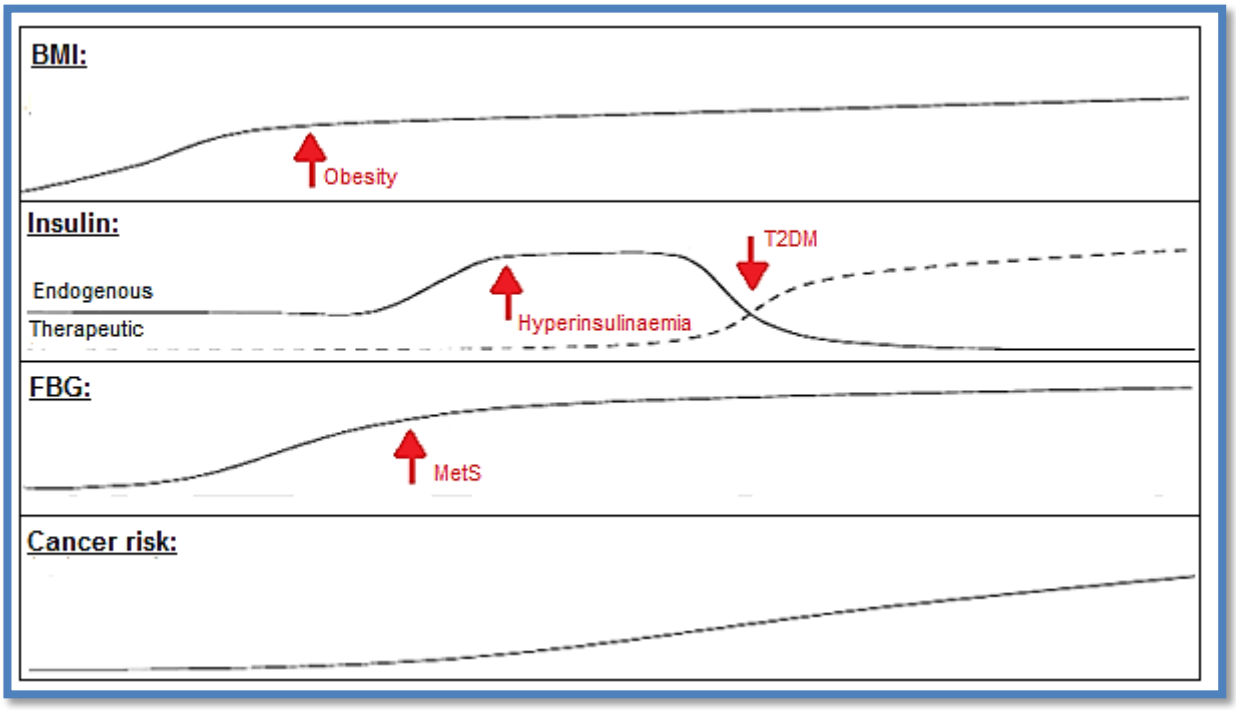

Figure 1.17: Components of the metabolic syndrome in relation to cancer risk, where increased body mass index, and blood glucose and insulin levels all contribute to an increased cancer risk.
(Adopted from: Westley & May, 2013).

1.6.2.1 Obesity

Many studies have shown that obesity is a major risk factor for several cancer types (Kuchiba *et al*., 2012; Leiba *et al*., 2012; Kawai *et al*.,2013; Wiedman *et al*., 2013; Li *et al*., 2014; Lindkvist *et al*., 2014; Roswall *et al*., 2014). Cancer cases were shown to be more frequently obese compared to being overweight (Leiba *et al*., 2012; Lindkvist *et al*., 2013; Wiedman *et al*., 2013; Pouchieu *et al*., 2014; Roswall *et al*., 2014). Furthermore, studies also reported an increased cancer risk with increasing BMI (summarised in Table 1.8).

Table 1.8: Association between different body mass index classes and cancer risk.

CANCER TYPE	BMI RANGE				REFERENCES
	Underweight BMI≤18.49 kg/m^2	Normal BMI=18.50-24.99 kg/m^2	Overweight BMI=25.00-29.99 kg/m^2	Obese BMI≥30.00 kg/m^2	
Female CNS				+	Wiedman *et al*., 2013
Female colorectal			+	+	Kuchiba *et al*., 2012
Female pancreatic				+	Johansen *et al*., 2010
Female thyroid		+	+	+	Almquist *et al*., 2015
Lung				+	Smith *et al*., 2012
Oesophageal	+				Kendall *et al.*, 2013; Lindkvist *et al*., 2014
Postmenopausal breast			+	+	Li *et al*., 2014; Kawai *et al*., 2013
Urethra			+		Leiba *et al*., 2012

Although various studies have shown an association between cancer risk and BMI, others reported no association between BMI and overall cancer risk, or the risk for breast or gastric cancers (Jaggers *et al*., 2009; Murff *et al*., 2011). A possible reason could include the specific area of fat deposition (Reeves *et al*., 2012; Comstock *et al*., 2014; Roswall *et al*., 2014; Cottet *et al*., 2015). Fat deposition, specifically VAT, was implicated in promoting pro-tumorigenesis through its effect on the production of inflammatory cytokines (IL-6 and tumour necrosis factor-α (TNF-α)), which causes a state of chronic low-grade inflammation (Donohoe *et al.*, 2012). Adipose tissue can act in both a paracrine and autocrine manner to promote the formation of cancerous lesions (Doyle *et al*., 2012). The less studied paracrine mechanisms include changes in the local production of inflammatory cytokines, adipokines, insulin, IGF-1, and growth factors, all of which have been shown to play a role in angiogenesis, cell proliferation, and apoptosis (Donohoe *et al*., 2011; Doyle *et al*., 2012). The autocrine or systemic effects of adiposity are well-studied, and include modifications to the insulin- and IGF-1 pathways, adipokine production, cancer cell signalling, and inflammatory pathways (Doyle *et al*., 2012). Commonly used measures to diagnose adiposity include WC, W:H, and SAD, all of which have been linked to certain cancer types, as shown in Table 1.9.

Table 1.9: Association between abdominal obesity and the associated cancer risk.

MEASURES OF ABDOMINAL OBESITY	CANCER TYPE
↑ **WC**	Colon
	Colorectal
	Female bladder
	Oesophageal
	Postmenopausal breast
↑ **W:H**	Colorectal
	Oesophageal
	Pancreatic
	Postmenopausal breast
↑ **SAD**	Oesophageal

(Adapted from: Reeves *et al*., 2012; Kendall *et al.*, 2013; Roswall *et al*., 2014; Comstock *et al*., 2014; Cottet *et al*., 2015).

Increased abdominal fat has been independently linked to the increased risk of several cancer types, including colorectal, breast, and oesophageal (Reeves *et al*., 2012; Comstock *et al*., 2014; Roswall *et al*., 2014; Cottet *et al*., 2015). Abdominal adiposity is also associated with several hormonal and metabolic alterations, such as IR, hyperinsulinaemia, T2DM, hypertension, and CVD (Jeon *et al*., 2011; Peer *et al*., 2013; Helelo *et al*., 2014). Adiposity in conjunction with the other risk factors for the MetS, further increases the risk of cancer development (Doyle *et al*., 2012). Although some studies reported that WC and HC measurements were not associated with either pancreatic or colorectal cancer risk, the combination of WC and HC in the W:H was associated with increased pancreatic, oesophageal, and colorectal cancer risk (Bracci, 2012; Kendall *et al.*, 2013; Cottet *et al*., 2015). Even though obesity is suggested to be the main contributor for cancer risk, the other risk factors of the MetS must also be explored.

1.6.2.2 Hypertension

Hypertension was shown to have different effects on overall cancer risk, as well as different types of cancer. An earlier study found that history of hypertension was associated with increased risk of cancer-related mortality (Lee *et al*., 2009a), whereas Jaggers *et al.* (2009) found no association between hypertension and cancer risk in men. A European study found that elevated BP (SBP above 140 mmHg and DBP above 90 mmHg) was not significantly associated with bladder cancer in women (Häggström *et al*., 2011), and no association between hypertension and breast or gastric cancer was also evident in women older than 65 years (Reeves *et al*., 2012; Lindkvist *et al*., 2013). Although hypertension may not be directly responsible for the increased risk of cancers, it may lead to cancer development through hormonal, inflammatory or growth factor pathways instead (Reeves *et al*., 2012).

1.6.2.3 Glucose intolerance and insulin resistance

History of T2DM was associated with an increased risk of cancer-related mortality (Lee *et al*., 2009a; Reeves *et al*., 2012; Rahman *et al*., 2015). Women were significantly more susceptible to develop pancreatic, gastric, or colon cancer if glucose levels were elevated (Johansen *et al.*, 2010; Wulaningsih *et al.*, 2012; Lindkvist *et al.*, 2013). Jaggers *et al.* (2009) found glucose to be responsible for an increased cancer risk of about 22.0 %. A specific association was also evident between HOMA-IR, insulin, and the risk of oesophageal cancer in older adults (mean age 61 years) (Duggan *et al*., 2013), whereas insulin levels were elevated in existing colorectal cancer patients (Ho *et al*., 2012). Table 1.10 summarises the association between cancer risk and the HOMA-IR, and glucose and insulin levels.

Table 1.10: Association between measures of glucose intolerance, insulin resistance, and the associated cancer risk.

MEASURES OF GLUCOSE INTOLERANCE AND IR	CANCER TYPE
↑ **glucose levels**	Pancreatic
	Gastric
	Colon
↑ **insulin levels**	Oesophageal
	Colorectal
↑ **HOMA-IR**	Oesophageal

(Adapted from: Johansen *et al*., 2010; Wulaningsih *et al.*, 2012; Ho *et al*., 2012; Duggan *et al*., 2013; Lindkvist *et al*., 2013).

High levels of trophic hormones, such as insulin and IGF-1, related to the MetS, have been proposed as possible mechanisms linking cancer, IR, and glucose intolerance (Jeon *et al.*, 2011; Erasmus *et al.*, 2012; Klug *et al.*, 2012; Camargo *et al.*, 2014; Lindkvist *et al.*, 2014). The IGF-1 is a critical component in cancer development, since it can promote cell proliferation, invasion, metastasis, drug resistance, and survival of tumour cells (Levine & Levine, 2014). Overexpression of the IGF-1R has also been reported in cancerous cells, most probably as a result of mutations that may occur in the tumour suppressor genes, thereby altering IGF-1 and insulin signalling pathways (Braun *et al*., 2011; Mendonça *et al*., 2015).

Circulating insulin levels affect IGF-1 levels through its downregulatory effect on IGFBP-1 and -2, thereby increasing the bioavailability of IGF-1 (Doyle *et al*., 2012). Both IGF-1 or -2 can bind to the IGF-1R that is located in the IGF-axis, which plays a role in cell proliferation, differentiation, and prevention of apoptosis (Doyle *et al*., 2012; Mendonça *et al*., 2015). The insulin receptor is identical to the IGF-1R, and these receptors are able to form heterodimers (IGF-1/IR-A) that control cell survival and -proliferation (Mendonça *et al*., 2015). In addition, elevated IGF-1 and insulin levels have been linked to receptor overstimulation, and eventually cancer development (Mendonça *et al*., 2015).

Research has recently indicated that IGF-1, which is essential for cell proliferation, growth, and metabolism, plays a pivotal role in IR and the MetS, and its link to cancer (Braun *et al*., 2011; Mendonça *et al*., 2015). Hyperinsulinaemia, one of the components of the MetS, may lead to increased cancer risk, since the effect of both hyperinsulinaemia and obesity can influence GH levels through the IGF-1 system (Braun *et al*., 2011; Doyle *et al*., 2012; Mendonça *et al*., 2015). Elevated IGF-1 levels have been associated with colorectal, oesophageal, lung, breast, and pancreatic cancers (Donohoe *et al*., 2010; Doyle *et al*., 2012). Additionally, an adverse lipid profile has also been linked with an increased cancer risk (Jaggers *et al*., 2009; Braun *et al*., 2011; Wulaningsih *et al*., 2012).

1.6.2.4 Dyslipidaemia

A study by Jaggers *et al*. (2009) found that elevated TG and reduced HDL-c levels each increased cancer risk by 25.0 %. Lee *et al*. (2009a) and Lindkvist *et al*. (2013) corroborated these results. A positive association was evident between TG, HDL-c levels, and oesophageal cancer risk, TG levels and colon cancer risk, and total cholesterol and rectal cancer risk (Wulaningsih *et al*., 2012). While no association was evident between total cholesterol levels and risk of gastric, and bladder cancer (Häggström *et al*., 2011; Lindkvist *et al*., 2013), the study by Wulaningsih *et al*. (2012) found an inverse relationship between HDL-c and gastric cancer risk. Table 1.11 summarises the correlation between several different cancer types with dyslipidaemia.

Table 1.11: Dyslipidaemia and associated cancer risk.

DYSLIPIDAEMIA	CANCER TYPE
↑ **LDL-c**	Oesophageal
↑ **TGs**	Colon
	Gastric
	Oesophageal
	Pancreatic
↑ **total cholesterol**	Rectal
↓ **HDL-c**	Gastric
	Oesophageal

(Adapted from: Johansen *et al*., 2010; Wulaningsih *et al*., 2012; Lindkvist *et al*., 2013).

Various mechanisms have been proposed to help explain the link between the altered lipid profile and increased cancer risk, including inflammation and lipid peroxides in lipoproteins that can induce cellular damage to promote carcinogenesis (Wulaningsih *et al*., 2012). Studies have shown that adipokines might also be related to cancer risk (Dalamaga *et al*., 2009; Ho *et al*., 2012; Mendonça *et al*., 2015).

1.6.2.5 Adipokines

Although the role of leptin is well studied in obesity, several of its other functions have recently been elucidated, which include its inflammatory response, suppression of gluconeogenesis and FA biosynthesis, its effect on insulin signalling and satiety sensation, as well as its role in carcinogenesis (Mendonça *et al*., 2015).

Elevated plasma leptin (hyperleptinaemia) and its circulating levels have been linked to adiposity, and cancer risk, a state known as leptin resistance (Doyle *et al*., 2012; Mendonça *et al*., 2015). A direct link between the MetS and cancer with regards to leptin is evident, because leptin stimulates human cancer cell growth (Mendonça *et al*., 2015). Although several studies have revealed the carcinogenic effects of leptin, human studies still lack the necessary evidence (Dalamaga *et al*., 2009; Ho *et al*., 2012). In addition, leptin resistance was associated with increased angiogenesis and cell proliferation, and neuroendocrine and pro-inflammatory effects (summarised in Figure 1.18) (Mendonça *et al*., 2015).

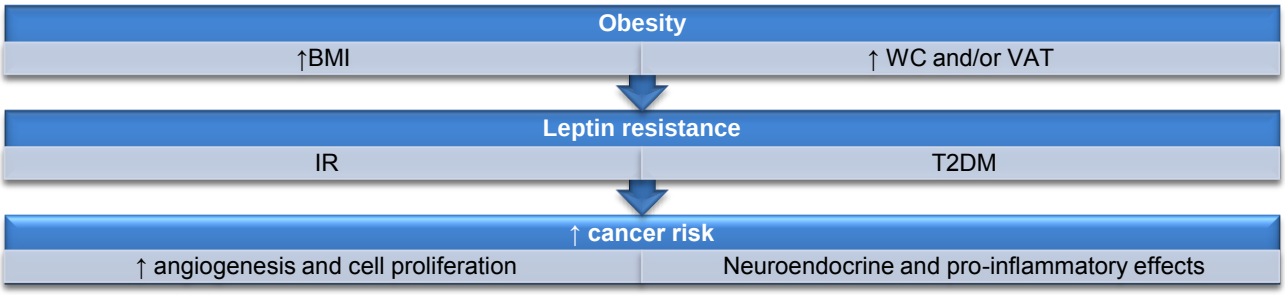

Figure 1.18: Obesity and its effect on cancer risk.
(Adapted from: Levine & Levine, 2014; Mendonça *et al*., 2015).

Adiponectin, on the other hand, has been shown to possess anti-carcinogenic properties by increasing apoptosis, and inhibiting inflammation, angiogenesis and cell proliferation (Donohoe *et al*., 2011; Levine & Levine, 2014). For example, adiponectin was shown to inhibit colorectal cancer cell growth, possibly due to its anti-inflammatory properties and its negative regulatory effects on angiogenesis (Braun *et al*., 2011). An earlier study by Dalamaga *et al.* (2009) found that women with pancreatic cancer had significantly higher adiponectin levels than controls; however, a more recent study found that adiponectin levels were not associated with colorectal cancer in women (Chandler *et al*., 2015). The discrepancies between these findings may be due to several factors, such as differences in gender and the associated fat deposition patterns, obesity, insulin sensitivity, and the adiponectin fraction measured (Shah *et al.*, 2012; Abu-Farha *et al.*, 2014; Chandler *et al*., 2015). Even though studies confirmed a relationship between the MetS and cancer, risk factors other than those responsible for the development of the MetS may be involved in the association between the MetS and the risk for cancer development.

1.6.3 Other factors linking metabolic syndrome to cancer

Various other risk factors can also influence the risk for cancer, including insufficient physical activity, unhealthy diet, increased alcohol consumption, tobacco use, ageing, and genetic predisposition (de Oliveira *et al*., 2012; Steyn *et al*., 2012; Hoebel *et al*., 2013; Miglani *et al*., 2015; Salonen *et al*., 2015; Strand *et al*., 2015). Figure 1.19 compared the number of cancer-related deaths in high- to middle- and low-income countries for some of the risk factors mentioned above. Tobacco use poses the greatest risk for cancer death, although largely in high-income countries, followed by increased alcohol consumption, and overweight and obesity (WHO, 2007; 2015a). In middle- and low-income countries, low fruit and vegetable intake, and alcohol consumption posed a significant risk for cancer-related deaths (WHO, 2007; 2015a).

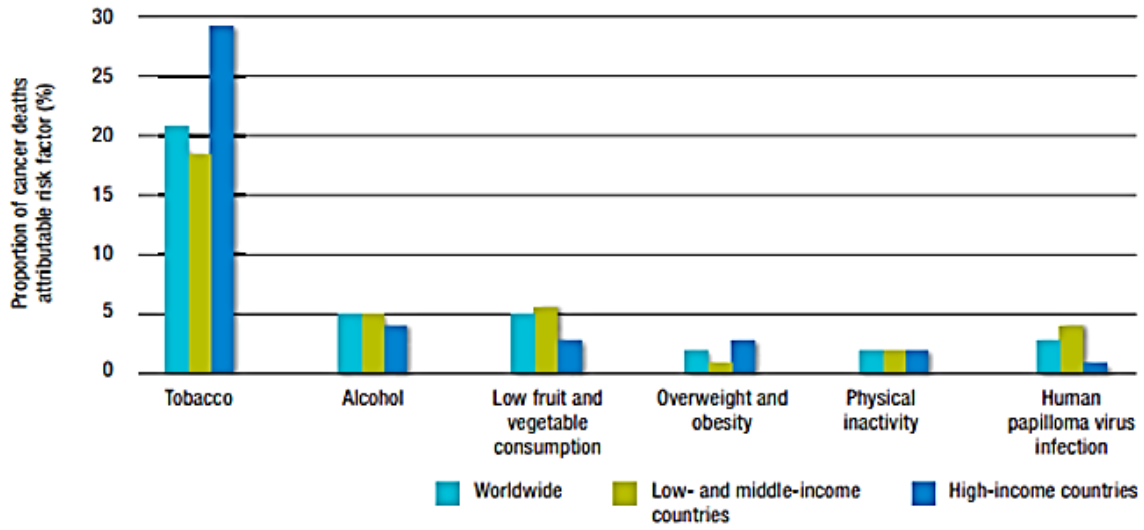

Figure 1.19: The effect of several risk factors on global cancer deaths in high-, and middle and low-income countries.
(Adopted from: WHO, 2007; 2015a).

The effect of each of the alternative risk factors on cancer risk will be discussed in the following sections.

1.6.3.1 Physical activity

Sedentary lifestyle is regarded as the fourth global cause of death (WCRFI, 2014). The WHO recommends that adults between the ages 18-64 years should partake in at least 150 minutes of moderate-intensity exercise, or 75 minutes of vigorous-intensity physical activity weekly (WHO, 2011). Hallal *et al*. (2012) estimated that 31.1 % of the global population were physically inactive, and that women tended to be more inactive compared to men (33.9 % *vs* 27.9 %). Interestingly, on the African continent, ~30.0 % of the population do not reach the recommended levels of physical activity (Hallal *et al*., 2012).

Anthropometric measures (BMI and WC) and body composition (percentage body fat) are inversely associated with sufficient physical activity, and decreases the risk of developing the MetS (Salonen *et al*., 2015; Strand *et al*., 2015). Physical inactivity and sedentary lifestyles have been shown to contribute an estimated six to ten percent of the NCDs, which includes coronary heart disease, T2DM, and breast and colon cancers (Lee *et al*., 2012).

Physical activity is also related to cancer risk, and this was illustrated in a study by Kawai *et al*. (2013), that reported a positive correlation between five or more hours of physical activity per week and reduced risk of developing breast cancer. Even low levels of physical activity have been associated with a decreased risk of breast, colon, colorectal, endometrial, postmenopausal breast, and pancreatic cancer (Ho *et al*., 2012; Miglani *et al*., 2015). In contrast, other studies have found that physical activity was unrelated to colorectal or CNS cancer (Wiedmann *et al*., 2013; Chandler *et al*., 2015). These contrasting results might be due to differences in inclusion criteria (men and women *vs* postmenopausal women), and the type of cancer studied.

The WHO reported that almost 50.0 % of South Africans were inactive, with women being more inactive than men (51.6 % *vs* 42.2 %) (WHO, 2010b). van Zyl *et al*. (2012) also reported that more urban (66.5 %) than rural (27.3 %) South Africans were inactive, since individuals in rural areas were more physically active by working as manual laborours and walking as a means of transport, compared to their urban counterparts, who often work indoors and use transport as opposed to walking. Increased urbanisation do not only lead to insufficient physical acitivity, but commonly also lead to changes in dietary patterns (Abrahams *et al*., 2011; Steyn *et al*., 2012; Shab-Bidar *et al*., 2014).

1.6.3.2 Diet

Different dietary types and the intake of specific food products have been related to increased or reduced cancer risk. Dietary variety have been shown to correlate with greater diet quality and intake of healthy food groups (fruit, vegetables, fibre, and dairy intake), and this is protective against the development of the MetS and lifestyle-associated cancers (de Oliveira *et al*., 2012; Song *et al.*, 2015). High salt intake, another contributor in the Westernised diet, is also associated with elevated BP, CVD, and gastric cancer (WCRFI, 2014). A strong association with colorectal cancer was also evident in studies employing the “Western diet”, a diet high in red and processed meat, animal fat, snack foods, and sugar sweetened beverages (including fruit juices), and low in dietary fibre (Theodoratou *et al*., 2013; Song *et al.*, 2015; Lucas *et al.*, 2016). Consumption of energy-dense, highly processed foods cause overweight and obesity, and this could lead to a multitude of other diseases, including cancer (Song *et al.*, 2015; Lucas *et al.*,

2016). Theodoratou *et al*. (2013) found that coffee intake is associated with reduced colorectal cancer risk, since it has anti-carcinogenic properties and increases motility in the large intestine. Not only can diet and coffee intake affect cancer risk, but smoking may also play a role.

1.6.3.3 Smoking

Smoking has mainly been associated with increased cancer risk; however, results have been inconsistent (Jaggers *et al*., 2009; Dalamaga *et al*., 2009; Johansen *et al*., 2010; Häggström *et al*., 2011; Murff *et al*., 2011; Ho *et al*., 2012; Kawai *et al*., 2013; Wiedmann *et al*., 2013; Pouchieu *et al*., 2014; Chandler *et al*., 2015; Cottet *et al*., 2015; Rahman *et al*., 2015). Differences between the findings of these studies may be attributed to several factors including underreporting of smoking habits, the specific cancer type studied, the effects of lifestyle factors such as alcohol intake, and metabolic factors such as obesity and IR/diabetes (Dalamaga *et al*., 2009). It is further suggested that smoking modifies the association between metabolic factors and cancer risk, although the extent of this is still unclear (Johansen *et al*., 2010). These studies also used different methods to quantify smoking, since some only differentiated between non-smoking and smoking, while others further differentiated between previous- and current smoking, the number of cigarettes smoked per day, and how long a person has been smoking. Furthermore, the respective studies included vast age ranges, from 20 to 88 years, and this may have contributed to the contrasting findings.

1.6.3.4 Age

It is well documented that age influences the development of the MetS (Hoebel *et al*., 2013; Clevenger *et al*., 2015). A recent study found that the prevalence of the MetS increased significantly in women between the ages 48 and 52 years, due to lower oestradiol levels, and redistribution of VAT associated with menopause (Hoebel *et al*., 2013; Clevenger *et al*., 2015; Strand *et al*., 2015). Furthermore, ageing was also associated with an increasing number of individual MetS risk factors (Hoebel *et al*., 2013). A recent local study found that the risk of female obesity increases significantly with age, since the prevalence of obesity was significantly higher in the older (older than 50 years) compared to the younger group (younger than 30 years) (prevalence of 58.5 % *vs* 20.0 %) (Senekal *et al*., 2015). The risk of hyperglycaemia, elevated HOMA-IR, and T2DM increase significantly with age (Leung *et al*., 2009; Erasmus *et al*., 2012; Frank *et al*., 2014; Strand *et al*., 2015). Similarly, hypertension was significantly higher in older (40-50 years) than younger (31-40 years) persons (Garrido *et al*., 2009; Gyakobo *et al*., 2012; Helelo *et al*., 2014).

A South African study determined the age-specific incidence of specific cancer types, and found that for oesophageal cancer, the incidence increases with age up to the maximum age of 69 years (Somdyala *et al*., 2010). The incidence of lung cancer was different to that of oesophageal cancer, since it increased rapidly between 35 and 39 years, and reached a maximum peak at 59 years of age (Somdyala *et al*., 2010). Additionally, studies have also found that different FAs may have pro- and anti-carcinogenic effects (Doyle *et al.*, 2012; Høstmark & Haug, 2013; Mendonça *et al*., 2015).

1.6.4 Possible link to fatty acids

Some FAs have anti-carcinogenic effects, and essential FAs can either inhibit or promote carcinogenesis (Høstmark & Haug, 2013). This effect is mainly thought to occur *via* the release of FFAs because of increased body weight, and its effect on IR, insulin levels, and ultimately the bioavailability of IGF-1 (illustrated in Figure 1.20). This process creates a pro-tumorigenic environment associated with reduced apoptosis and increased cell proliferation (Boden, 2009; Doyle *et al.*, 2012; Mendonça *et al*., 2015).

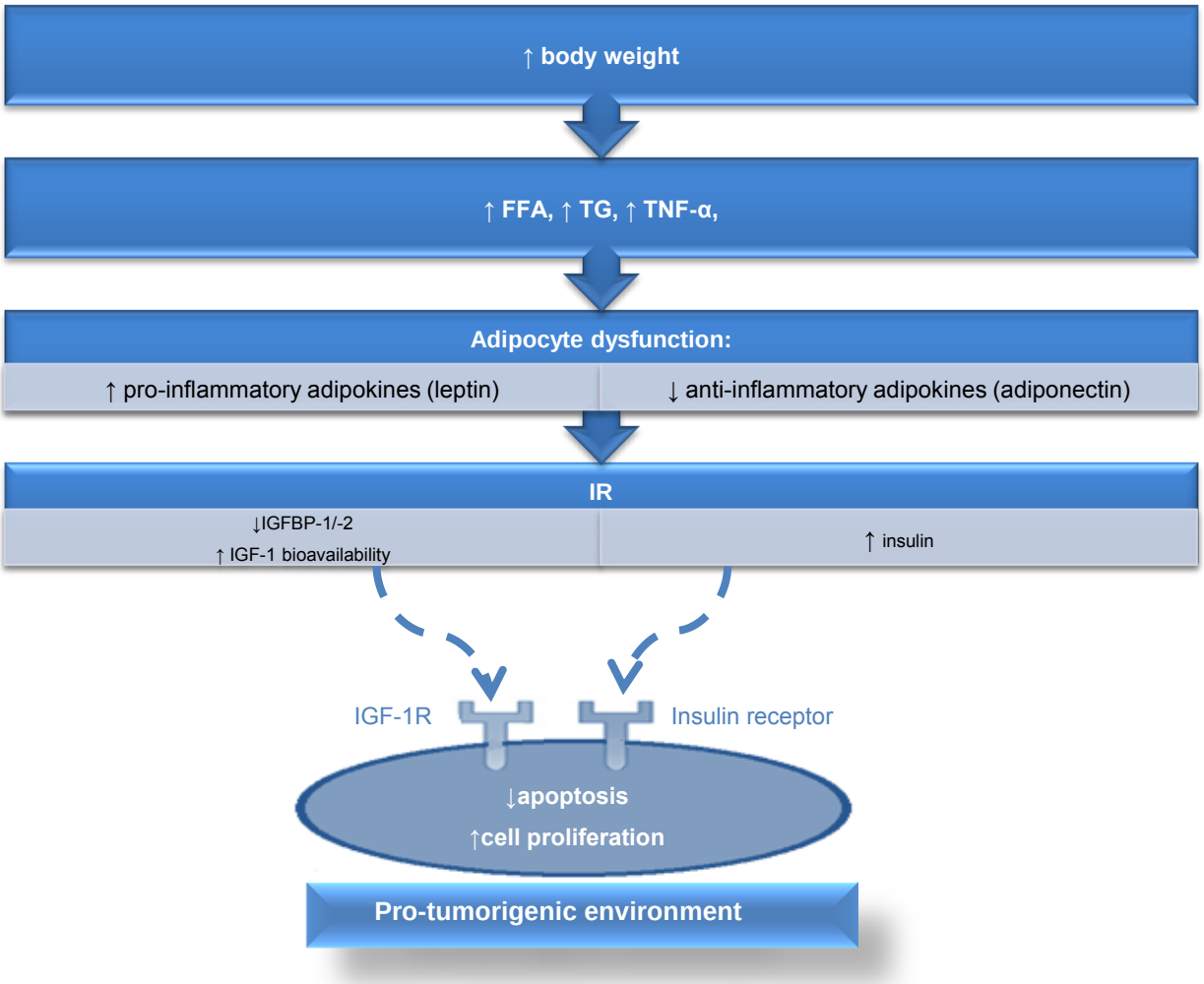

Figure 1.20: Diagrammatic representation of the association between fatty acids and carcinogenesis. (Adapted from: Boden, 2009; Mendonça *et al*., 2015).

Some FAs also have unfavourable effects on cancer risk, and may possibly play a role in the metabolic alterations that are ultimately associated with cancer, although contrasting results have been obtained (Anderson & Ma, 2009; Shannon *et al.,* 2010; Pouchieu *et al*., 2014). Studies by Mikirova *et al*. (2004) and Shannon *et al.* (2010) found that SA and PA levels of cancer patients were significantly lower compared to healthy controls, while a more recent study by Pouchieu *et al*. (2014) found an inverse association between SFAs and overall cancer risk. However, a 2015 study found that SFA levels were not associated with the risk of colorectal cancer (Cottet *et al*., 2015). The difference in study design, specific cancer type studied, and the blood pool used for sampling to determine FA levels may explain these conflicting results.

Furthermore, Mikirova *et al*. (2004) suggested that carcinogenesis is associated with alterations in lipid metabolism, since tumour cells exhibit decreased desaturase activity, which was evident from the lower SA and higher OA levels seen in cancer patients. Although the exact mechanisms are still unclear, desaturase activity is also under hormonal and nutritional control, and these factors may have played a role in the conflicting results (Mikirova *et al*., 2004; Pouchieu *et al*., 2014). The study by Mikirova *et al*. (2004) also found that OA and PLA levels were higher in cancer patients compared to healthy controls, and in addition, Kojima *et al*. (2005) found that MUFAs were positively associated with colorectal cancer risk.

The equilibrium of the different FAs to each other is essential for good health, and for this reason the SA to OA ratio (SA:OA), or n-9 SI was established as an indicator of cancer risk (Jackson *et al.*, 1997; Chavarro *et al*., 2013). An earlier study found that the n-9 SI was a predictor of postmenopausal breast cancer, and even though the exact role of the n-9 SI in cancer is still unclear, it was shown that unsaturated FAs enhance membrane fluidity, with a higher n-9 SI being protective against malignancies (Mikirova *et al*., 2004; Shannon *et al.*, 2010).

Polyunsaturated FAs have shown both pro- and anti-carcinogenic effects, possibly by affecting gene expression, alteration of signal transduction molecules involved in cell growth, angiogenesis, and metastasis (Hussein, 2013). A study by Pouchieu *et al*. (2014) found that total PUFA levels were directly associated with overall cancer risk. The total PUFA levels include the Ω-3 and Ω-6 PUFAs, which have pro- and anti-inflammatory effects respectively, and this may explain the contrasting associations with cancer risk (Strandvik, 2011; Hussein, 2013).

An earlier study by Bagga *et al.* (2002) found that the total Ω-3 PUFAs were inversely associated with breast cancer risk in women, which was corroborated by Kojima *et al*. (2005) for colorectal cancer risk and Pouchieu *et al*. (2014) for overall cancer risk. A study by Kuriki *et al.* (2007) also found that the total Ω-3 PUFAs were inversely associated with gastric cancer risk,

because the Ω-3 PUFAs, EPA and DHA, are said to play roles in competitive inhibition against tumour development. When specifically looking at individual Ω-3 PUFAs, one study found a weak negative association between DPA and colorectal cancer risk (Kojima *et al*., 2005). Omega-3 FAs have demonstrated to have beneficial effects on carcinogenesis, since Ω-3 FAs provide substrates for lipid peroxidation, while lipid peroxidation is inversely associated with cell division and tumour growth (Mikirova *et al*., 2004; Anderson & Ma, 2009). Several mechanisms exist describing the beneficial effects of Ω-3 FAs on carcinogenesis, including lipid peroxidation, eicosanoid synthesis alterations, enhanced immunity, intercellular communication, membrane fluidity, intercellular communication, and hormone secretion (Mikirova *et al*., 2004). Figure 1.21 illustrates some of the anti-carcinogenic effects of the Ω-3 PUFAs.

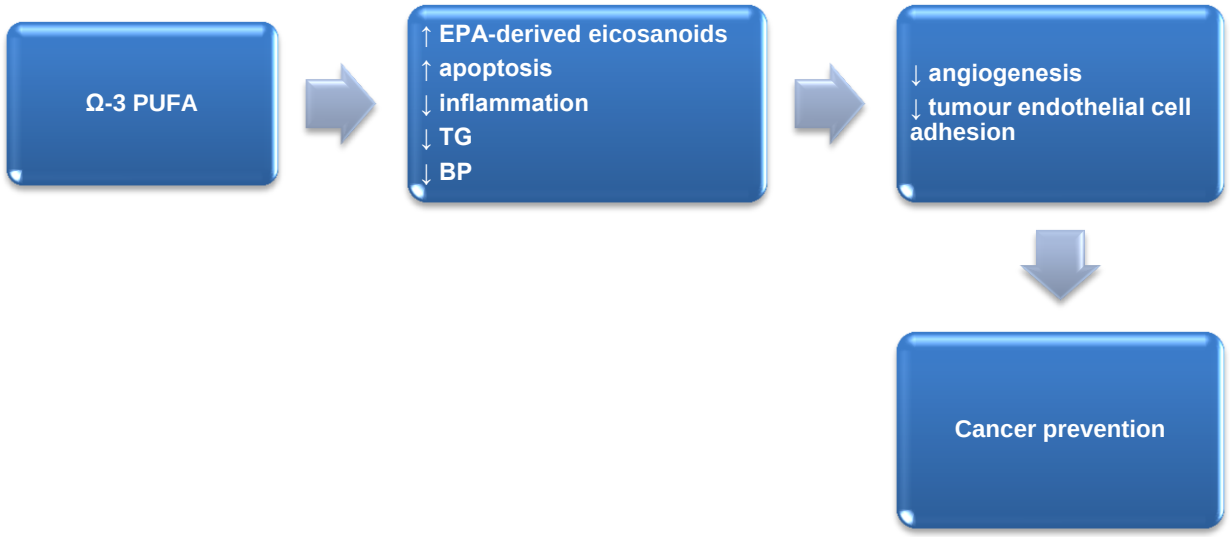

Figure 1.21: Proposed mechanism whereby omega-3 fatty acids can prevent cancer.
(Adopted from: Hussein, 2013).

Increasing Ω-6 PUFA levels has also been associated with cancer risk, in particular breast cancer risk (Murff *et al*., 2011). However, the risk of colorectal cancer was not associated with the total Ω-6 levels of abdominal SAT samples (Cottet *et al*., 2015). Overall cancer risk was inversely related to dihomo-γ-linolenic acid (DGLA, 20:3n-6) levels (Pouchieu *et al*., 2014), although Cottet *et al*. (2015) found that DGLA levels were positively related to colorectal cancer risk in women older than 45 years. Another study, this time comparing plasma DGLA levels of female participants (mean age 51.3 years) with and without cancer, found that the DGLA levels of the former group were lower (Pouchieu *et al*., 2014). Results on the effects of Ω-6 FAs on cancer risk however, remain inconclusive and further research is needed to establish a relationship between Ω-6 FA levels and cancer risk (Anderson & Ma, 2009). It is also evident from this section that FAs are associated with cancer risk, although the exact mechanisms are still unclear.

1.7 SUMMARY AND SIGNIFICANCE OF THE STUDY

The prevalence of the MetS and other metabolic-associated diseases are on the rise, particularly in developing countries, and this is a major concern as it poses a risk for developing CVD, T2DM, and cancer (Alberti *et al.*, 2009; Jeon *et al.*, 2011; Erasmus *et al.*, 2012; Klug *et al.*, 2012; Hoebel *et al*., 2013; Camargo *et al.*, 2014). Childhood obesity rates are also on the increase, leading to a pattern of overweight children becoming obese adults (Doyle *et al*., 2012). The MetS is associated with alterations in lipid- and FA metabolism that leads to an adverse FA profile, which is associated with an increased risk of the individual components of the MetS, and the MetS itself (Shab-Bidar *et al.*, 2014; Žák *et al.*, 2014).

Findings suggest that the development of several lifestyle-associated cancers is also on the increase in developing countries, and this is affected by the increasing prevalence of the MetS (Lindkvist *et al*., 2014; Almquist *et al*., 2015; Miglani *et al*., 2015). In South African adults, obesity and inadequate physical activity are the greatest cancer risk factors (WHO, 2014c). Several studies established a strong relationship between obesity, dietary fat intake (more specifically the effects of individual FAs), the components of the MetS, and cancer risk (Kuchiba *et al.,* 2012; Leiba *et al*., 2012; Reeves *et al*., 2012; Kawai *et al*., 2013; Wiedman *et al*., 2013; Li *et al*., 2014; Lindkvist *et al*., 2014; Almquist *et al*., 2015).

This MetS is related to a multitude of metabolic-related diseases and cancers that lead to high morbidity and mortality rates, which ultimately affects socioeconomic factors (WHO, 2010a). A proper understanding of the relationship between the MetS and cancer risk may have a significant clinical and economic influence on health systems, since cancer is a common cause of death worldwide. The financial and social NCD-burden in resource-limited settings debilitates public health care systems, because the burden of communicable diseases (such as human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), tuberculosis (TB), and malaria) is on the increase (Crowther & Norris, 2012). Currently, the prevalence of the MetS is largely unknown in the Western Cape, and consequentially, MetS-associated cancer risk is undetermined. Patients with the MetS are often in a state of chronic low-grade inflammation, which cause the release of high levels of trophic hormones (including insulin and IGF-1), and these are related to elevated cancer risk (Blüher & Mantzoros, 2015). Genetic-, lifestyle- (dietary habits, smoking, and physical inactivity), metabolic-, and environmental factors all play a role in the aetiology of the MetS, which warrants further research (Steyn *et al*., 2012).

Findings suggest a direct link between obesity, the MetS, and cancer risk, since adipose tissue is influenced by dietary intake (Shab-Bidar *et al*., 2013). This necessitates the evaluation of the

relationship between dietary intake and adipocyte dysfunction. Although various studies have focussed on the MetS in a South African setting, limited studies investigated the association of FA profiles, the MetS, and possible link to cancer risk in different South African ethnic groups. This study may also provide baseline data for future studies on the long-term effect of the MetS on nutritional status and cancer risk.

Since the present study uses additional parameters (adipose tissue biomarkers, FA levels, *etc*.) to describe the MetS, this may add value to future development of new or existing clinical definitions for different South African ethnic groups. Lastly, it is important to raise awareness among South Africans concerning lifestyle choices to steer the nutrition transition into a more positive direction in an attempt to delay the onset of various chronic diseases.

1.8 AIM

The present study therefore aimed to describe the association and interaction between the MetS and specific FAs and FA ratios, anthropometric measures, dietary intake, and a possible link to cancer risk in a female population from the Western Cape Province, South Africa.

1.9 OBJECTIVES

The following objectives were identified:

- To determine the prevalence of the MetS in a female farm worker population in the Stellenbosch Municipal region of the Western Cape Province, South Africa;
- To determine the possible associations between specific RBC membrane FAs and FA ratios, anthropometric measures, dietary intake, and cancer risk in female participants with and without the MetS;
- To study the association between specific RBC membrane FAs and FA ratios, anthropometric measures, dietary intake, and cancer risk in participants according to different BMI classes in these different groups.

CHAPTER 2: MATERIALS & METHODS

2.1 INTRODUCTION

This chapter will describe research materials, as well as methods used to collect data. Eligibility criteria of participants, study variables, and the statistical measures used to determine variables are also outlined and explained.

2.2 ETHICAL CONSIDERATIONS

Ethical clearance was obtained from the Human Research Ethics Committee (HREC) of Stellenbosch University (N13/04/052) (Appendix A). This research project was conducted in accordance with the principles of the Declaration of Helsinki (2000), Good Clinical Practice, and the constitution of South Africa. All relevant parties were contacted and consulted prior to the initiation of any data collection.

Different farming areas around Stellenbosch were approached and the necessary information about the study was communicated to possible participants. All interested participants signed up, where after research visits were arranged on different days in the weeks to follow. Two nurse practitioners and the operational manager of the Owethu Clinic on the Villiera farm assisted in arranging appointments on Tuesdays and Thursdays, whereas staff at the Solms-Delta wine estate (outside of Franschoek) and Neethlingshof wine estate (outside of Stellenbosch) arranged appointments on Wednesdays and Fridays respectively.

On the day of data collection, the aims and objectives of the study were thoroughly explained to each participant in Afrikaans, English or isiXhosa (with the help of a translator) prior to volunteers signing the informed consent form (Appendix B). A copy of the information leaflet with all relevant information regarding the purpose and procedures of the study was handed to each participant to read through in their own time. Participants were made aware of any possible risks and benefits in partaking in the study, although no major risks were anticipated. All participants had the right to withdraw from the study at any point in time, even after signing the informed consent form. Throughout the data collection process, the participants were often asked whether they were still interested in taking part in the study. All participants were also reassured of their anonymity throughout the study. All raw data collected were coded, preventing any links to names and personal details. After the participants agreed to participate, the informed consent form was signed, after which data collection commenced.

2.3 STUDY DESIGN

Initially, a cohort study was planned to determine the effect of urbanisation on the association and interaction between the MetS, anthropometric measures, dietary intake, and a possible link to cancer risk in a female population from different areas in the Western Cape Province, South Africa. However, due to financial constraints, the study design was changed to a descriptive cross-sectional study that was conducted around the Stellenbosch farming areas in the Boland district, Western Cape Province, South Africa (Figure 2.1). Data collection took place at the Owethu Clinic of the Pebbles Project Health Program (situated on the Villiera wine estate just outside of Stellenbosch), Neethlingshof wine estate (Stellenbosch) and Solms-Delta wine estate (Franschoek) from March to July 2015.

Figure 2.1: Regional map of the Cape Winelands area indicating the location of Stellenbosch.
(From: https://www.google.co.za/maps/place/Stellenbosch/).

2.4 STUDY POPULATION

The sample population consisted of female farm workers between the ages of 20 and 60 years that were permanent residents of the Stellenbosch surrounding area (Boland district). All participants were recruited from March to July 2015.

2.4.1 Samples size

The sample size was calculated using a power analysis test with the help of a biostatistician. This study focused on the assessment of mean values for metabolic-associated blood parameters, RBC membrane FAs, IGF-1 and leptin levels, specific anthropometric measurements, and three-day dietary intakes. A model for participant recruitment was designed

for statistical power to be reached to indicate statistically significant differences between participant groups. Using Equation 2.1 with the confidence interval set at 5 %, the sample size was calculated.

$$n = \frac{1.96^2 \sigma^2}{d^2}$$

Equation 2.1: Formula to calculate sample size.
(Israel, 1992).

A total of n=24 participants per group were needed to determine mean values for metabolic-associated blood parameters, RBC membrane FAs, IGF-1 and leptin levels, anthropometric measurements and dietary intakes with 80 % power. In total, the following number of participants was needed per group:

- n=24 females with the MetS and overweight according to BMI classification;
- n=24 females with the MetS and obese according to BMI classification;
- n=24 females without the MetS and normal weight according to BMI classification;
- n=24 females without the MetS and overweight according to BMI classification;
- n=24 females without the MetS and obese according to BMI classification.

This gave a total study population of n=120 participants. To account for possible incomplete datasets or other difficulties, an additional ten percent was calculated to give a final sample size of n=132 participants.

Although a convenient sample was chosen from the Stellenbosch farming areas, the study population was specifically chosen to represent a population that might be in a nutrition transition. A random sample of n=80 was drawn from the total female study population recruited (n=145) to exclude possible selection bias based on the five groups mentioned above.

2.4.2 Recruitment of participants

All volunteering participants were recruited at the respective sites according to the following inclusion and exclusion criteria:

Inclusion criteria:

- Participants had to be of South African descent from any ethnic group, and permanent residents of the Western Cape Province;
- Participants had to be female farm workers between the ages 20 to 60 years;
- Participants were only included if they agreed to participate by signing the informed consent form.

Exclusion criteria were:

- Participants that were not of South African descent and not permanent residents of the Western Cape Province;
- Participants that were younger than 20 years of age, and/or older than 60 years of age;
- Participants who did not agree to partake, or withdrew their consent.

Once the participants gave written informed consent, data collection commenced. This specific sample population was chosen to provide information on the prevalence of the MetS, and furthermore to describe the effects of the MetS and its components on RBC membrane FAs, IGF-1 and leptin levels, and its possible association to cancer risk. Participants were first screened for the MetS using the IDF criteria (IDF, 2006). This definition stated that a person is diagnosed with the MetS if they have an increased WC ≥80 cm (females) and any two of the following criteria:

- Fasting plasma glucose ≥5.6 mmol/L, impaired fasting glucose or T2DM;
- TG levels ≥1.7 mmol/L;
- HDL-c levels ≤1.3 mmol/L (females);
- BP ≥130/≥85 mmHg or treatment for hypertension.

The total study sample population (n=191) included both genders, of which n=42 were males. From the remaining female sample population (n=149), n=four were excluded, and the remainder of the group (n=145) categorised into either the MetS (n=68), or non-MetS (n=77) groups. Each of these groups were then subdivided according to BMI values into either normal weight (BMI=18.5-24.9 kg/m^2), overweight (BMI=25.0-29.9 kg/m^2) or obese (BMI≥30.0 kg/m^2) categories (WHO, 2004a; NHANES, 2011) (see Figure 2.2).

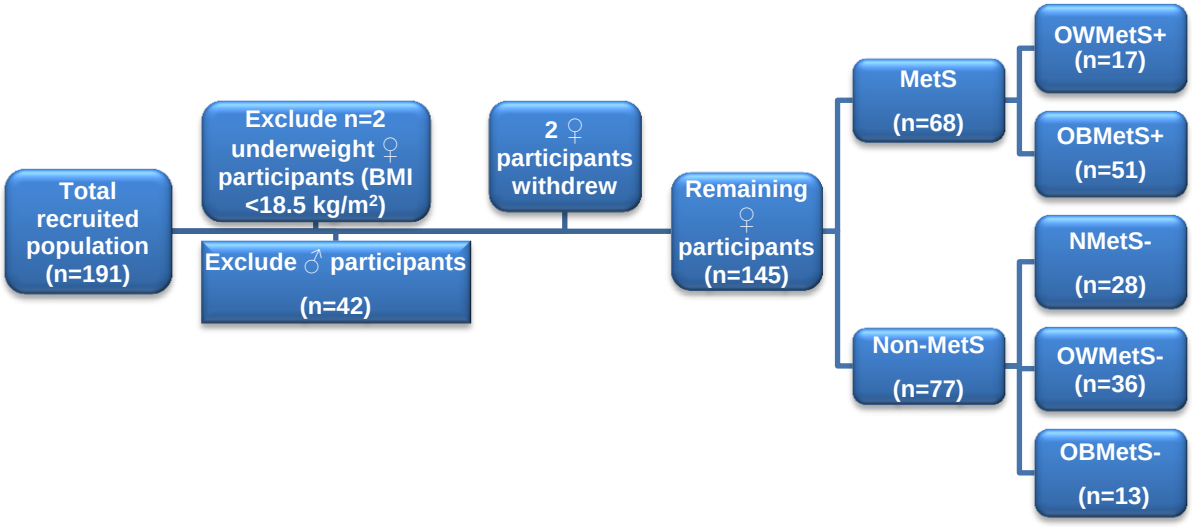

Figure 2.2: A diagram explaining the exclusion of participants and group allocation of total participants recruited.

After females were classified into their respective groups, a random sample was drawn from each group (with the exception of two groups) to yield a total of n=80. From the n=68 that presented with the MetS, a random sample of n=34 were drawn. In this group, n=17 females were overweight (OWMetS+) and n=17 were obese (OBMetS+). Forty-six females were randomly selected from the non-MetS group, sixteen of which presented with a normal BMI (NMetS-), n=17 were classified as overweight (OWMetS-) and n=13 were obese (OBMetS-). For a full description of the final study population, refer to Figure 2.3.

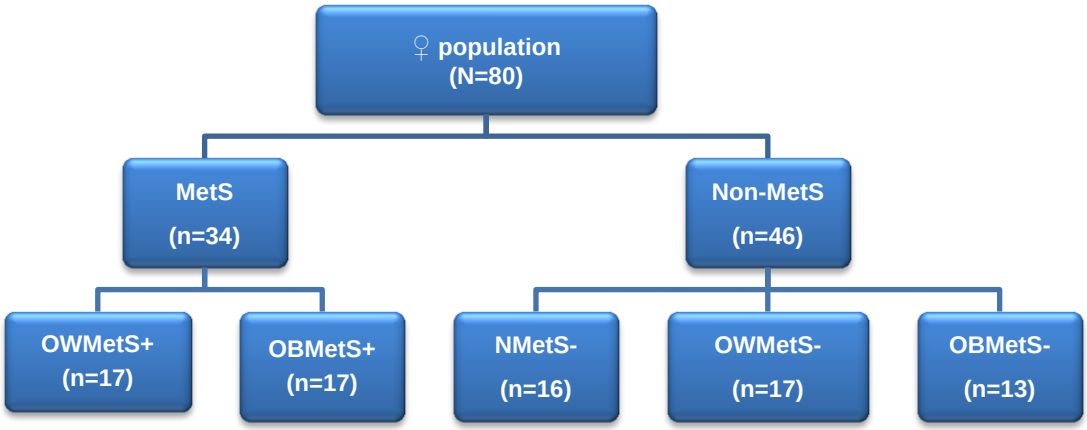

Figure 2.3: The random allocation of participants into the different study groups.

2.5 DATA COLLECTION

Each research visit was scheduled in order to collect data from between ten to twelve participants. All data sheets were numbered and each participant received a unique number for the entire data collection process. The following procedures were subsequently followed:

2.5.1 Systolic-, diastolic blood pressure and heart rate

After signing consent, volunteers were seated in a comfortable chair for five to ten minutes before BP was measured, twice on the right arm using an aneroid sphygmomanometer (Erka Perfect Aneroid 48, Germany) and stethoscope (Littmann 3M stethoscope, United States of America). Heart rate was also assessed during this procedure using a finger pulse plethysmograph (Contec medical systems, China). Participants who showed severe hypertension during this assessment were referred to the nursing staff for medical treatment.

2.5.2 Haematology

All blood sampling was performed by a registered phlebotomist using sterile procedures. The venous blood sample was collected from the cubital vein in the right arm using a vacutainer or sterile butterfly infusion set (BD Vacutainer® Safety-Lok™, Becton, Dickinson and Company, United Kingdom). Blood was collected into two serum separating tubes (2x4 mL SST), one

plasma sodium fluoride (1x4 mL) and two plasma ethylenediaminetetraacetic acid (2x4 mL EDTA) tubes. The EDTA tubes were immediately centrifuged at 4 000 revolutions per minute (rpm) for ten minutes, followed by separation of the plasma from the blood cells. The SST tubes were left to clot for ten minutes at room temperature (22 °C) and then centrifuged. The serum from one of the SST tubes and the plasma from the two EDTA tubes were aliquoted into Eppendorf tubes and immediately placed on dry ice for later analyses. The RBCs from one of the EDTA tubes were wash-treated according to a specific protocol (section 2.5.2.1) and placed on dry ice for RBC FA analysis (Opperman & Benade, 2013). All serum and plasma samples were stored at -80 °C for IGF-1 and leptin analysis, once transferred from the dry ice to a bio-freezer at the Department of Physiological Sciences' analytical laboratory.

The remaining SST tube and the sodium fluoride tube (required no centrifugation) were stored at four degrees Celsius until transport to the local PathCare laboratory for subsequent analysis. A fasted lipogram profile (total cholesterol, LDL-c, HDL-c, and TG), and insulin were determined from the SST tube, whereas glucose was determined from the sodium fluoride tube. The MetS status was then determined by evaluating WC, BP, HDL-c, glucose and TG levels (IDF, 2006), and participants were categorised into the different study groups.

2.5.2.1 Fatty acid profile analyses

The EDTA tube containing the RBCs was washed three times with sterile 0.9 % saline, followed by a four minute spin at 2500 rpm between each of the wash steps (Opperman & Benade, 2013). During each of these steps, care was taken to remove the buffy coat (white blood cells, WBCs) together with the saline, to render a WBC-free RBC sample for FA analysis. After the final removal of the saline, the RBCs were immediately frozen on dry ice in the EDTA tube.

Fatty acid analyses were done at the Functional Foods Research Unit at Cape Peninsula University of Technology (CPUT). On the day of analysis, chloroform-methanol (2:1 v/v) was used until complete phase separation was achieved. Following centrifugation (at 2 000 rpm for four minutes), the lower chloroform phase was transferred into transmethylation tubes and evaporated to dryness under a nitrogen stream in a 50-60 ˚C water bath. This was followed by the transesterification of FAs to fatty acid methyl esters (FAMEs), where bound FAs were hydrolysed and methylated simultaneously with toluene and methanol sulphuric acid for two hours at 70 °C.

Distilled water and hexane were added, and after shaking and settling, the hexane layer containing the FAMEs (top layer) were transferred to a second set of transmethylation tubes and the FAMEs evaporated to dryness under a nitrogen stream in a 50-60 ˚C water bath. A small

quantity of hexane (100 µL) was added, followed by mixing, whereafter the remaining sample was transferred into specific gas liquid chromatography (GLC) vials for analysis using GLC (GLC, Thermo, Focus) equipped with a flame ionisation detector and BPX 70 capillary column (oven temperature: three degrees Celcius per minute from 160 to 220 °C) (Bligh & Dryer, 1959; Jackson *et al*., 1997; Harris, 2009; Opperman & Benade, 2013) (see Appendix C for the full protocol). For FA identification and analysis, the peaks on the chromatograph were identified based on the known retention times of commercial standards. Results were expressed as the relative percentage of the total FA area.

The main FA fractions of interest for this specific study included: (a) the Ω-3 index (EPA+DHA); (b) n-9 SI (SA:OA) and (c) Ω-3:Ω-6 (EPA+DHA:LA+AA). The desaturase activity, an indicator of FA metabolism, was also estimated using FA product-to-precursor ratios of individual FAs to calculate (d) Δ-9 SCD 1 (PLA:PA) and (e) Δ-9 SCD 2 (OA:SA).

2.5.2.2 Insulin-like growth factor-1 analyses

Serum samples for IGF-1 analysis were transferred from the -80 °C bio-freezer unit to a -20 °C freezer one day before the IGF-1 enzyme-linked immunosorbent assay (ELISA) (Abcam ELISA^{TM}, ab100545, United Kingdom). This ELISA uses an affinity tag labelled capture antibody and a reporter conjugated detector antibody that immunocaptures the sample analyte in solution.

A thorough literature search, based on populations similar to that of our study, revealed that serum samples had to be diluted 20x (Yee *et al*., 2010; Bogin *et al*., 2015; Koeglenberg *et al.*, 2015). For assay procedures, a 100 ng/mL IGF-1 stock standard was prepared, followed by serial dilutions to yield the following standards necessary for generating the standard curve: 30 ng/mL, 12 ng/mL, 4.8 ng/mL, 1.92 ng/mL, 0.768 ng/mL, 0.307 ng/mL and 0.123 ng/mL. Samples and standards were added in duplicate to the respective wells and incubated for two and a half hours at room temperature (22 °C). The plate was washed thoroughly and a biotinylated IGF-1 detection antibody was added, followed by incubation for a further hour. Following another wash step, horseradish peroxidase (HRP) was added to the plate and incubated for 45 minutes. The plate was washed again, and 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution added and incubated in a dark section of the analytical laboratory for 30 minutes. The stop solution was then added to terminate the colour reaction and a proportional signal was generated to the amount of bound analyte, after which its intensity was measured using a universal microplate reader (EL800, Bio-tek Instruments, Inc. Analytical & Diagnostic Products, United States of America) at a wavelength of 450 nm (see Appendix D1 for the complete protocol).

2.5.2.3 Leptin analyses

The serum leptin analyses were performed using a commercially available ELISA kit (Abcam SimpleStep ELISA™, ab179884, United Kingdom). Exploring scientific literature, based on leptin levels of similar populations, indicated that all serum samples had to be diluted 40x for samples from normal and overweight females, and 90x for obese participants regardless of their MetS status (de Castro *et al*., 2015; García-Jiménez *et al*., 2015; Jafari-Vayghan *et al*., 2015). Initially, a 2 500 pg/mL leptin stock standard solution was prepared, followed by serial dilutions to yield the following standards: 750 pg/mL, 375 pg/mL, 187.5 pg/mL, 93.8 pg/mL, 46.9 pg/mL, 23.4 pg/mL and 11.7 pg/mL. These standards were used to construct the standard curve in order to determine the leptin concentration. Following sample dilution, the samples and standards were added to the plate wells in duplicate. An antibody cocktail was added to each microplate well and the plate was incubated for one hour at room temperature (22 °C) using a laboratory shaker (Stovall life science incorporated, United States of America) on a gentle shaking cycle.

After incubation, the plates were washed three times to remove any unbound material and subsequently a TMB substrate was added to each well. The plate was incubated for another ten minutes in a dark section of the analytical laboratory area on a gentle shaking cycle. The stop solution was then added and a change in colour from blue to yellow was noted. The samples were analysed using a universal microplate reader (EL800, Bio-tek Instruments, Inc. Analytical & Diagnostic Products, United States of America) at a wavelength of 450 nm (see Appendix D2 for the complete protocol).

2.5.3 Anthropometric measurements

All anthropometric assessments were performed by a level one technician anthropometrist using standardised and calibrated equipment (Stewart & Sutton, 2012). All data collected were entered in a structured International Society for Advancement of Kinanthropometry (ISAK) proforma Microsoft Excel data sheet in duplicate or triplicate (Appendix E) (Stewart & Sutton, 2012). Anthropometric assessments were done using technical error of measurement (TEM), which entailed a third measurement in cases where the second measurement differed more than five percent from the first. The same standard equipment was used throughout the study for all measurements.

Participants were informed of the measurements being done and what was expected of them. All participants were instructed to remove any loose and heavy items from their pockets (Stewart & Sutton, 2012). All measurements were done on the right side of the body, unless a volunteer presented with any physical abnormality. The following anthropometric measures were taken:

2.5.3.1 Base measurements: weight and stretched stature

Participants were instructed to remove all heavy clothing, shoes, hair ornaments, and hats. Participants were weighed to the nearest 0.01 kilograms using a SECA™ 634 digital electronic scale (Seca United Kingdom, Birmingham, England). The scale was placed on a flat surface and the volunteer was then asked to stand in the centre of the platform with their weight evenly distributed between the feet, and the hands at the sides.

Stretched stature was obtained using a portable Leicester™ stadiometer (Leicester, England) to the nearest 0.5 cm. Volunteers were positioned with their head in the Frankfort plane with the feet together. When measuring height, the participant's head, shoulders, buttocks, calves and heels were in contact with the measuring rod (Figure 2.4). With the participant in this position, the Broca plane of the stadiometer was gently pressed down until it made contact with the vertex of the head. Volunteers were instructed to inhale and hold their breath until the measurement was taken, after which they were instructed to exhale. During the inhalation, slight upward pressure to the head was applied to ensure maximum stretched stature (NHANES, 2011; Stewart & Sutton, 2012).

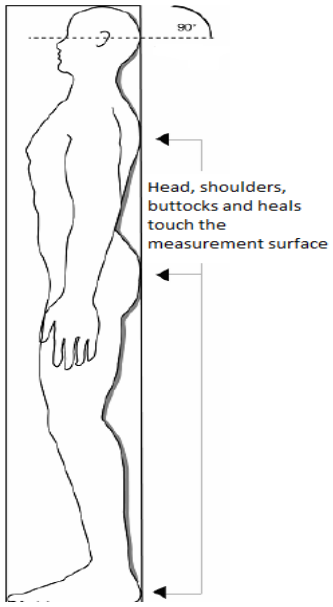

Figure 2.4: The correct position to measure stretched stature.
(NHANES, 2011).

2.5.3.2 The body mass index

The BMI (kg/m^2) was calculated using the standard equation of weight divided by the height squared (Jarvis, 2011; Willet & Hu, 2013). The volunteers' BMIs were recorded and they were then classified according to the WHO (2004a) guidelines (Table 2.1):

Table 2.1: Classification of the body mass index.

CLASS		BMI (kg/m^2)
Underweight	Severe malnutrition	<16.00
	Moderate malnutrition	16.00-16.99
	Mild malnutrition	17.00-18.49
Normal range	Normal	18.50-24.99
Overweight	Pre-obese	25.00-29.99
Obese	Obese class I	30.00-34.99
	Obese class II	35.00-39.99
	Obese class III	≥40.00

(WHO, 2004a).

2.5.3.3 Waist- and hip circumferences, waist-to-height ratio and waist-to-hip ratio

The WC, HC, W:Ht, and W:H were used to indirectly assess abdominal obesity (IDF, 2006; Anunciação *et al*., 2014; Cheong *et al*., 2015). These measures served as surrogates used to indirectly assess subcutaneous- and visceral fat tissue distribution (NHANES, 2011). All circumferences were measured using a flexible and inelastic Lufkin tape measure (Lufkin, United States of America). Both the WC and HC measurements were done with the participant standing with their feet evenly spread apart and arms relaxed to the sides. The participants were instructed to abduct their arms to allow the tape measure to be passed around the abdominal region (Stewart & Sutton, 2012).

The WC measurement was assessed to the nearest 0.1 cm at the level of the lower 10^{th} costal rib and superior iliac crest on the narrowest part of the abdomen using the cross-handed technique (Figure 2.5). The measuring tape was held parallel to the floor and tight, ensuring that the skin was not pinched (WHO, 2008; Jarvis, 2011; Stewart & Sutton, 2012).

Figure 2.5: Correct position of the tape measure for the waist- and hip circumference measurements.
(Adopted from: Jarvis, 2011).

Different WC measurements are associated with anticipated health risks. In women a WC of 80 cm or more is associated with an increased risk of metabolic diseases, whereas a WC of 88 cm or more is associated with a substantially increased risk of such diseases (IDF, 2006; WHO, 2008). The HC was measured at the level of the greatest posterior protuberance, perpendicular to the long axis of the trunk, and included bony structures, muscle and fat, although this measurement tend to reflect the muscle component (WHO, 2008; Willet & Hu, 2013).

To further assess the distribution of VAT, the WC, HC and height were respectively used to estimate the W:H and W:Ht. A normal W:H is <0.85, while a W:H ≥0.85 increases the risk of metabolic diseases (WHO, 2008).

2.5.3.4 Sagittal abdominal diameter

The Holtain Kahn sagittal abdominal calliper (Holtain Kahn, United Kingdom) was used to determine the external distance between the abdomen and the lumbar vertebrae at the level of the iliac bone. Participants were instructed to lie in the supine position on an examination bed with the knees at a 90° angle with their feet resting on the bed. The participants were then asked to raise their hips in order to slip the lower arm of the calliper underneath the back of the participant. The shaft of the calliper was then adjusted in a vertical manner while the upper arm was moved to barely touch the participant's abdomen (Figure 2.6) (Stewart & Sutton, 2012).

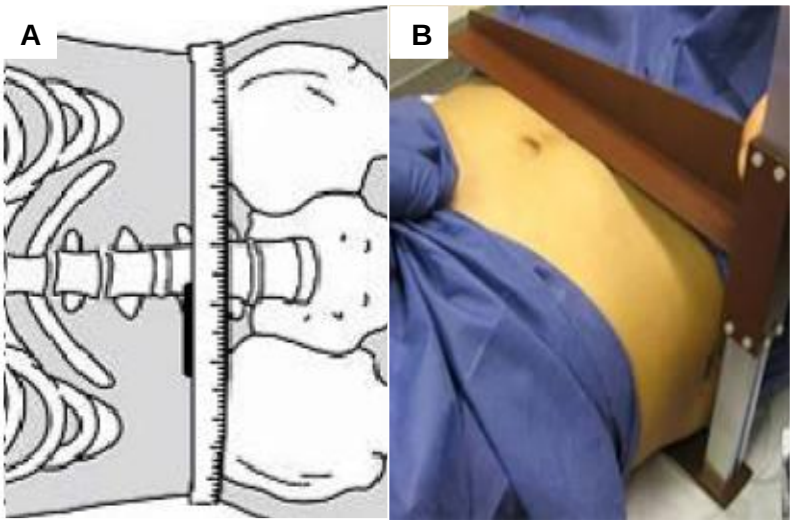

Figure 2.6: Correct measurement of the sagittal abdominal diameter by A) determining the iliac level and B) alignment of the upper arm of the calliper with the iliac.
(Adapted from: NHANES, 2011; Stewart & Sutton, 2012).

Before taking the reading, it was ensured that the lower arm of the abdominal calliper was aligned with the participants' upper arm. The mean of at least two SAD measurements were recorded in this manner (NHANES, 2011; Stewart & Sutton, 2012).

2.5.3.5 Bioelectrical impedance analysis

Bioelectrical impedance analysis was performed using the BioScan920-II multi-frequency (5 kHz, 50 kHz, 100 kHz and 200 kHz) analyser (Maltron BioScan 920II, United Kingdom), which passes a small electric current through the body when connected to the electrodes (Appendix F) (Bodystat, United Kingdom Ltd). Prior to the BIA measurement, a pre-test protocol had to be followed for all volunteers. Ensuring no electrical interference occurred; participants were instructed to remove any jewellery, metal objects, cell phones or other electronic equipment prior to the test. Participants were also instructed to empty their bladders prior to testing.

Impedance measurements for the SAT were done by placing the electrodes as illustrated in Figure 2.7. Using the umbilicus as reference point, electrodes labelled channel 2 and 4 were placed seven cm to either side of the umbilicus. From here, electrodes labelled channel 1 and 3 were placed five cm from the electrodes from channel 2 and 4.

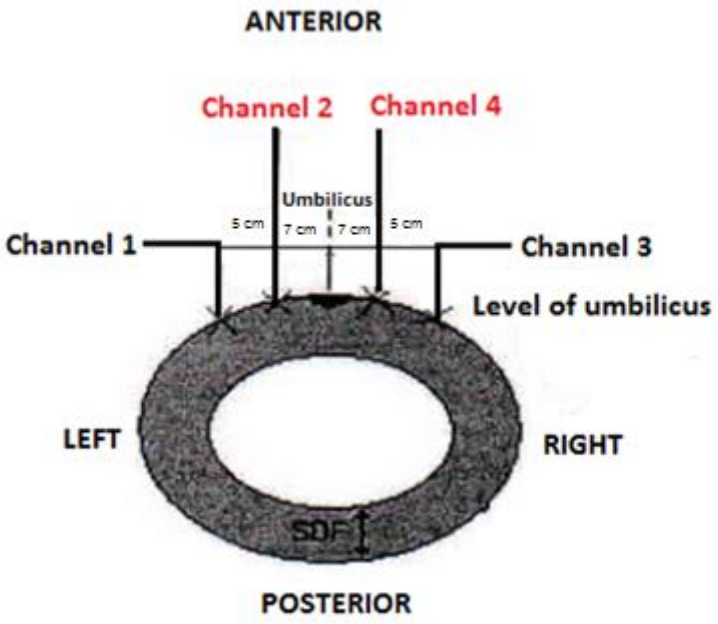

Figure 2.7: Correct placement of the electrodes for the subcutaneous adipose tissue impedance measurement. Channels 2 and 4 are red and channels 1 and 3 are black.
(Maltron BioScan 920II Manual, 2003).

For the assessment of VAT, the diameter (d on Figure 2.8) was calculated as the WC (in cm) divided by eight. Channel 1 was placed just above the umbilicus. The distance (d) was then measured to the left-hand side of the participant where channel 2 was placed on the same level of the abdomen as that of channel 1. Channel 3 was placed in the small of the back in line with

channel 1 and 2. Again, the distance (d) was measured to the left of the participant where channel 4 was placed.

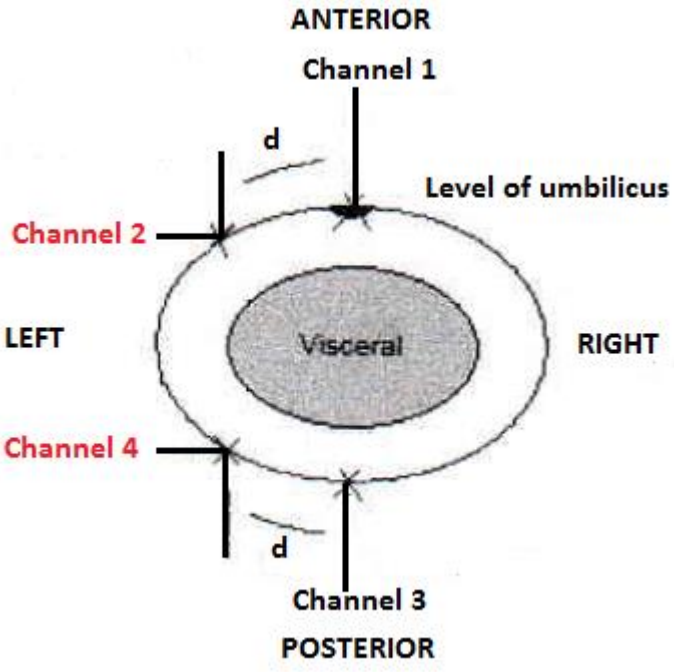

Figure 2.8: Correct placement of the electrodes for the visceral adipose tissue impedance measurement. Colour of electrodes 1 to 4 is indicated by the label colour. The distance "d" is calculated at the waist circumference (in cm) divided by eight.
(Maltron Bioscan 920II Manual, 2003).

For both SAT and VAT measurements, the phase angle and impedance measurements were recorded from the Maltron BioScan 920 BIA machine. These values together with the WC were entered into the Maltron BioScan 921 v1.1 software programme that calculated the SAT and VAT areas (in cm^2), VAT:SAT and respective VAT and SAT percentages. Furthermore, the software also determined the adiposity status according to VAT and SAT distribution of the participant by classifying them into either the normal, overweight and obese categories.

2.5.4 Three-day food diary

A three-day food diary assessment tool was used to quantify food intake over a three-day period of time (Appendix G). All questionnaires were available in Afrikaans or English, and a translator from the local community was used for isiXhosa-speaking participants where needed. Each volunteer was introduced to the food record and after a thorough explanation; they were instructed to record any food or beverages that were consumed over a three-day time period following data collection. Each participant also received a leaflet with all relevant information of the three-day food records, including an example of a food record.

Accuracy of dietary intake was established by recording the time any food or beverage was consumed, the type of meal (breakfast, in between, lunch, snack, dinner), what product was

consumed, the recipe of the product (*e.g.* was milk or sugar added), and the amount that was consumed (half a cup or a full cup). The Food Photo Manual (N.P. Steyn and M. Senekal, 2004) was used for life-sized sketches of food portions, food types, and food preparation methods. Household measures (cups, mugs, plates, *etc*.) and two-dimensional life-size drawings of foods were used to estimate portion sizes. Participants were encouraged to provide complete homemade recipes where possible to improve the accuracy of the dietary intake data. Completed three-day food records were then collected from participants and entered in the password-protected FoodFinder^{TM}3 software program (MRC, FoodFinder III). The FoodFinder^{TM}3 software program is able to use coding to convert household measures to grams to ultimately quantify the intake of specific macro- and micronutrients (energy, protein, carbohydrate, fat, vitamin and mineral intakes).

2.5.5 Questionnaires

Several studies have indicated that different lifestyle factors may influence metabolic- and biochemical parameters, anthropometric measurements, body composition, and ultimately risk of CVD and cancer (Steyn *et al*., 2012; Lindkvist *et al*., 2014; Almquist *et al*., 2015; Miglani *et al*., 2015). For this reason, each participant also completed questionnaires for familial cancer history, smoking/tobacco use, alcohol consumption, and physical activity.

2.5.5.1 Familial cancer history

An immediate family member with cancer history may also influence cancer risk of other family members (Bracci, 2012; Lindkvist *et al*., 2014). Consequently, the familial cancer history questionnaire of the current study included questions regarding the relation of the cancer patient to the participant, type of cancer, current status of cancer, and treatment of the cancer (Appendix H1).

2.5.5.2 Lifestyle factors

The current study used a standardised questionnaire to assess smoking/tobacco use, and alcohol consumption (Appendix H2). Participants were asked to indicate if they were non-, previous- or current smokers and drinkers.

The Global physical activity questionnaire (GPAQ) was used to indirectly assess physical activity of study participants (Appendix H3) (WHO, 2004b). The WHO developed the GPAQ to assess physical activity in epidemiological studies using a standardised questionnaire. The GPAQ consists of different sections with questions relating to activities at work, travelling

(walking or using a bicycle), recreational activities (sports, fitness or leisure), and sedentary behaviour. Physical activity is also assessed according to intensity (moderate or vigorous), duration, and frequency.

2.6 DATA MANAGEMENT

The consent forms, which included the participant names and linking codes, were kept in a lockable storage cabinet in the data storage room at the Department of Physiological Sciences at Stellenbosch University, separate from the rest of the data files. All other data files were also stored in this lockable cabinet. Only the principle investigator and co-investigator had access to the original records and data. A sign in and sign out system was used during data capturing.

Data entry in Microsoft Excel 2010 was done on the day of data collection to minimise inconsistencies and missing data. All data were entered on the premises of the Department of Physiological Sciences. Data were cleaned and also cross-checked to ensure accuracy. Checking of the data was done by verifying the data in the Microsoft Excel data sheet with the participant data files. All data entered were stored under a password-protected system on the main frame of Stellenbosch University, which is saved and backed up daily. The database was saved on two separate password-protected computed systems for security purposes.

2.7 STATISTICAL ANALYSIS

The Microsoft Excel database was exported to Statistica version 12 (Statistica Version 12. Copyright© StatSoft, 2007 Southern Africa-Research (Pty) Ltd) for statistical analysis. Statistical analysis was performed with the help of a biostatistician at Stellenbosch University.

Normality was assessed both graphically (by using box-and-whisker plots and histograms) and statistically (using Shapiro-Wilk tests). Descriptive statistical analysis was done for the entire study population, where after group demographics together with clinical characteristics were summarised and compared. For nominal data, the student t-tests were used to indicate differences between two groups if the data were normally distributed, whereas Mann-Whitney U tests were performed for abnormally distributed data.

In order to compare more than two groups, a factorial analysis of variance (ANOVA) was used together with a Bonferroni *post hoc* test to assess the significance of the differences found. Pearson' correlations coefficients were calculated to determine possible associations between variables. For all analyses, a $p<0.05$ was considered statistically significant, and the mean±SEM were calculated for all parameters reported.

CHAPTER 3: RESULTS

3.1 INTRODUCTION

This chapter describes the study population, as well as the different study groups. Initially, the descriptive profile of the study population is provided, followed by a comparison of the non-MetS and the MetS groups. Lastly, the groups were compared according to metabolic status and BMI. The anthropometric (BMI, W:H, W:Ht, SAD) and metabolic-associated parameters, BIA characteristics, RBC membrane FA profile, biochemical analyses for IGF-1 and leptin, and dietary intake are also provided as part of each section. The chapter concludes with relevant relationships between some of the physiological parameters of interest.

3.2 DESCRIPTIVE PROFILE OF THE STUDY POPULATION ACCORDING TO METABOLIC STATUS

The study population consisted of n=80 volunteering female participants between the ages of 20 and 60 years, with a mean age of 37.2±1.2 years. Using IDF criteria (IDF, 2006), a total of n=34 (42.5 %) MetS participants were identified and n=46 (57.5 %) participants were recruited into the non-MetS group (Figure 3.1).

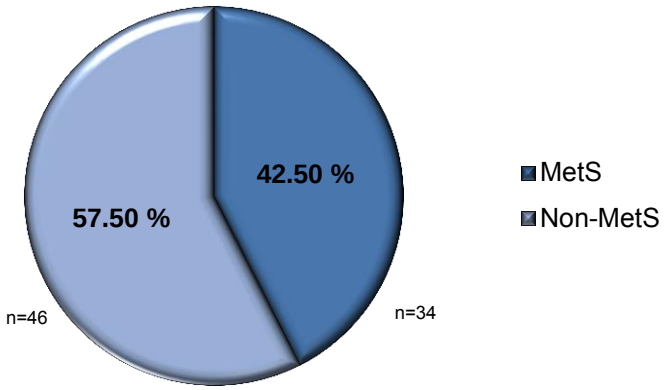

Figure 3.1: Proportion of study participants from the total study population, with (n=34) and without (n=46) the metabolic syndrome.

3.2.1 Distribution and prevalence of the metabolic syndrome risk factors

Since the MetS consists of several individual components, the distribution of participants that displayed one, two, three, four, and five risk factors were determined, as well as the prevalence of each of these individual components. For the MetS group, three risk factors were most common (n=20, 58.8 %), whereas an equal number of participants presented with four (n=7, 20.6 %), and five (n=7, 20.6 %) risk factors. The majority of the participants in the non-MetS group presented with two risk factors (n=25, 54.3 %), followed by one risk factor (n=14, 30.4 %).

Six participants (13.0 %) also had no risk factors for the MetS, and one non-MetS participant presented with three risk factors (2.2 %) (Figure 3.2).

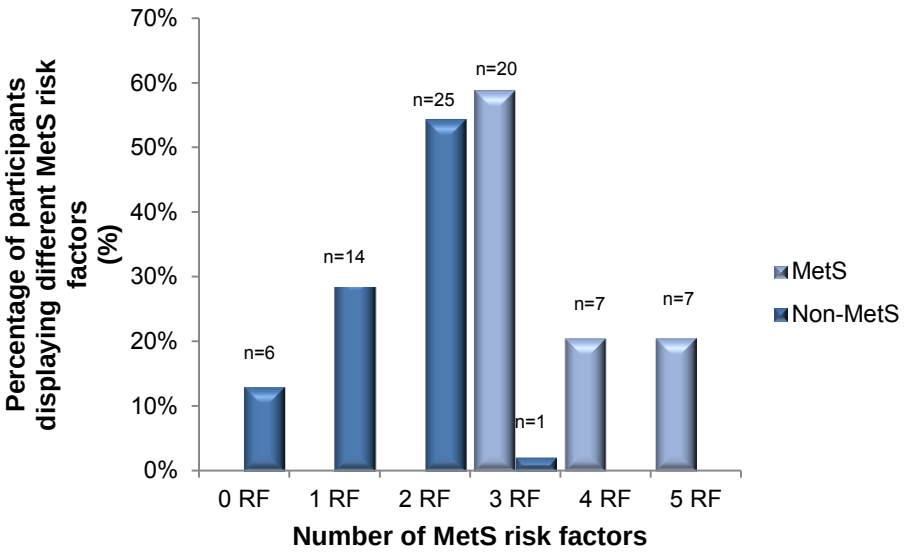

Figure 3.2: Distribution of risk factors according to the IDF criteria for participants diagnosed with (n=34) and without (n=46) the metabolic syndrome.

The prevalence of each of the MetS risk factors differed between the two groups. For the MetS group, abdominal obesity (n=34, 100 %) was predominant, with an almost equal number of participants presenting with hypertension (n=28, 82.4 %) and low HDL-c levels (n=26, 76.5 %). For the non-MetS group, hypertension (n=22, 47.8 %) was the most common risk factor, followed by abdominal obesity (n=18, 39.1 %), and low HDL-c levels (n=16, 34.8 %). The prevalence of hypertriglyceridaemia (n=4, 8.7 %) and hyperglycaemia (n=4, 8.7 %) was similar in the non-MetS group. These two MetS components were also shown to be less prevalent in the MetS group (n=19, 55.9 % for hypertriglyceridaemia, and n=14, 41.2 % for FBG) (Figure 3.3).

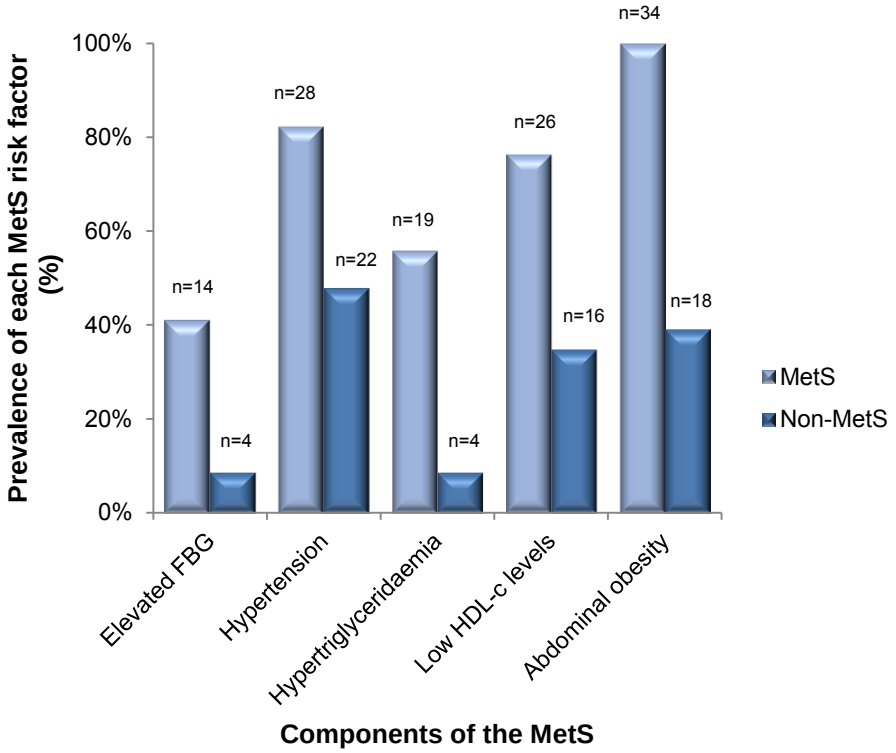

Figure 3.3: Prevalence of the individual metabolic syndrome-associated risk factors for participants diagnosed with (n=34) and without (n=46) the metabolic syndrome.

3.2.2 Anthropometric and metabolic-associated blood parameters

The participants from the MetS group were significantly older compared to the non-MetS participants (35.0±1.5 *vs* 40.2±1.8 years, $p<0.05$). Anthropometric measures from participants in the MetS group appeared to be significantly higher compared to the non-MetS group for weight (81.4±2.6 *vs* 70.9±2.9 kg, $p<0.05$), BMI (32.6±1.0 *vs* 28.7±1.1 kg/m^2, $p<0.05$), and WC (91.9±1.8 *vs* 79.6±1.8 cm, $p<0.001$).

Significant differences were also observed for SBP (141.9±3.2 *vs* 128.5±2.3 mmHg, $p<0.001$) and DBP (89.3±2.3 *vs* 82.7±1.6 mmHg, $p<0.05$) in the MetS and non-MetS groups. Similarly, participants in the MetS group showed significantly elevated blood glucose (6.1±0.6 *vs* 4.7±0.1 mmol/L, $p<0.05$) and TG levels (2.0±0.2 *vs* 1.1±0.1 mmol/L, $p<0.001$), whereas HDL-c was significantly lower (1.1±0.0 *vs* 1.4±0.1 mmol/L, $p<0.001$). Furthermore, the participants in the MetS group also presented with significantly higher insulin (34.4±5.1 *vs* 15.6±2.0 mIU/L, $p<0.001$) and LDL-c levels (3.2±0.2 *vs* 2.7±0.2 mmol/L, $p<0.05$), compared to those from the non-MetS group.

Several other anthropometric indices for adiposity were also determined, including HC, W:H, W:Ht and SAD (Table 3.1). Both the W:H (0.8±0.0 *vs* 0.8±0.0; $p<0.001$) and W:Ht (0.6±0.0 *vs*

0.5±0.0, p<0.001) were significantly higher in the MetS group, as was the SAD (25.4±0.5 *vs* 22.6±0.6 cm, p<0.001). Similarly, a statistical trend was also noted for HC (111.1±2.1 *vs* 104.7±2.4 cm, p=0.06). Table 3.1 indicates the basic participant characteristics, metabolic-associated parameters and anthropometric measures of the MetS and the non-MetS groups.

Table 3.1: Descriptive characteristics, including anthropometric and metabolic-associated blood parameters for the MetS and the non-MetS groups.

VARIABLE	MetS (n=34)	Non-MetS (n=46)	P-VALUE *
Age (years)	40.24±1.80	34.96±1.45	p<0.05
Height (m)	1.58±0.01	1.57±0.01	ns
Weight (kg)	81.43±2.63	70.88±2.93	p<0.05
BMI (kg/m^2)	32.59±0.97	28.66±1.07	p<0.05
WC (cm)	91.90±1.80	79.60±1.76	p<0.001
SBP (mmHg)	141.88±3.18	128.54±2.29	p<0.001
DBP (mmHg)	89.29±2.29	82.72±1.55	p<0.05
Heart rate (b/m)	83.18±2.30	79.39±2.27	ns
Blood glucose levels (mmol/L)	6.11±0.57	4.66±0.12	p<0.05
Insulin levels (mIU/L)	34.42±5.13	15.61±1.98	p<0.001
HDL-c levels (mmol/L)	1.05±0.03	1.36±0.05	p<0.001
LDL-c levels (mmol/L)	3.20±0.15	2.70±0.16	p<0.05
TG levels (mmol/L)	2.04±0.19	1.08±0.10	p<0.001
HC (cm)	111.14±2.07	104.68±2.43	p=0.06
W:H	0.83±0.01	0.76±0.01	p<0.001
W:Ht	0.58±0.01	0.51±0.01	p<0.001
SAD (cm)	25.44±0.54	22.56±0.62	p<0.001

* All values presented as mean±standard error of the mean (SEM). ns indicates not significant.

3.2.3 Bioelectrical impedance analysis characteristics

Bioelectrical impedance data, presented in Table 3.2, indicated that the VAT area was significantly greater in the MetS compared to the non-MetS group (343.4±3.3 *vs* 283.6±10.7 cm^2, p<0.001). The SAT % (27.6±1.1 *vs* 33.1±1.1 %), and VAT % (72.4±1.1 *vs* 66.9±1.1 %), and therefore the VAT:SAT (2.8±0.2 *vs* 2.2±0.1) were also significantly different between these two groups (p<0.001 for all).

Table 3.2: Bioelectrical impedance data for visceral- and subcutaneous adipose tissue for the MetS and non-MetS groups.

VARIABLE	MetS (n=34)	Non-MetS (n=46)	P-VALUE *
SAT area (cm^2)	217.29±7.35	174.35±7.68	ns
VAT area (cm^2)	343.38±3.25	283.57±10.67	p<0.001
SAT %	27.56±1.11	33.08±1.11	p<0.001
VAT %	72.44±1.11	66.92±1.11	p<0.001
VAT:SAT	2.82±0.15	2.20±0.12	p<0.001

* All values are presented as mean ± standard error of the mean (SEM). ns indicates not significant.

3.2.4 Red blood cell membrane fatty acid profile

No FAs or FA ratio differences were observed between the MetS and non-MetS groups (Table 3.3).

Table 3.3: Red blood cell membrane fatty acid levels for the total population categorised according to metabolic syndrome status.

VARIABLE		MetS (n=34)	Non-MetS (n=46)	P-VALUE *
Individual FAs (%)	16:0 (PA)	18.83±0.63	17.32±0.81	ns
	16:1n-7 (PLA)	0.28±0.02	0.26±0.02	ns
	18:0 (SA)	9.45±0.29	8.92±0.39	ns
	18:1n-9 (OA)	9.24±0.35	8.86±0.44	ns
	18:2n-6 (LA)	8.86±0.40	8.50±0.43	ns
	18:3n-3 (α-LA)	0.02±0.01	0.05±0.01	ns
	20:4n-6 (AA)	11.37±0.44	10.71±0.52	ns
	20:5n-3 (EPA)	0.74±0.07	0.76±0.08	ns
	22:5n-3 (DPA)	1.67±0.06	1.59±0.08	ns
	22:6n-3 (DHA)	5.93±0.35	5.80±0.34	ns
FA ratios	Ω-3 index	6.67±0.40	6.55±0.40	ns
	Ω-3:Ω-6	0.34±0.02	0.35±0.02	ns
	Δ-9 SCD 1	0.01±0.00	0.01±0.00	ns
	Δ-9 SCD 2	0.98±0.04	0.98±0.03	ns
	n-9 SI	1.10±0.07	1.12±0.08	ns

* All values presented as mean±standard error of the mean (SEM). ns indicates not significant.

3.2.5 Biochemical blood analysis for insulin-like growth factor-1 and leptin

From the total sample (n=80), n=14 (17.5 %) participants displayed IGF-1 levels below the detection limit (n=three from the MetS group, and n=11 from the non-MetS group), whereas n=17 (21.3 %) participants had leptin levels below the detection limit (n=four from the MetS group, and n=13 from the non-MetS group). An additional n=four (5.0 %) participants' IGF-1 levels (n=two from the MetS group, and n=two from the non-MetS group), and n=three (3.8 %) participants' leptin levels (n=two from the MetS group, and n=one from the non-MetS group) were excluded from the statistical analysis because the concentrations were too high (outliers) and skewed the data.

Insulin-like growth factor-1 from the remaining participants (n=62) were significantly higher in the MetS group (103.1±17.6 *vs* 53.9±7.2 ng/mL, $p<0.05$), whereas leptin levels (n=60) were significantly lower (684.9±97.7 *vs* 1119.0±162.7 pg/mL, $p<0.05$) (Table 3.4).

Table 3.4: Insulin-like growth factor-1 and leptin levels for participants with and without the metabolic syndrome.

VARIABLE	MetS	Non-MetS	P-VALUE *
IGF-1 concentration (ng/mL) ▲	103.14±17.55	53.92±7.16	p<0.05
Leptin concentration (pg/mL) ▪	684.92±97.66	1118.98±162.67	p<0.05

* All values presented as mean±standard error of the mean (SEM).
▲ MetS (n=29, 46.8 %) and non-MetS (n=33, 53.2 %).
▪ MetS (n=28, 46.7 %) and non-MetS (n=32, 53.3 %).

3.2.6 Familial cancer history

Data on familial cancer history revealed that n=27 (33.8 %) participants either had a mother, father, or sibling with cancer (Figure 3.4). From the n=27, n=five (5.3 %) participants reported familial cancer history from more than one family member. Two participants had a mother and father with cancer history, while three participants had a father and sibling with cancer history.

Among the participants with familial cancer history, breast cancer was the most common cancer type.

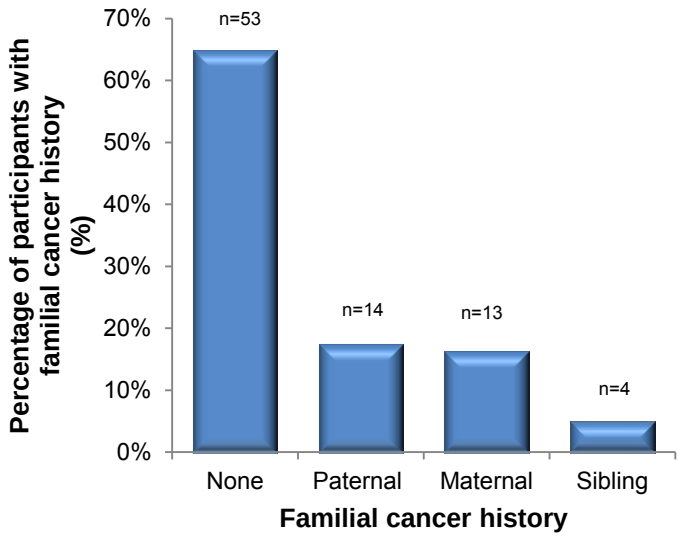

Figure 3.4: The percentage of participants with no familial cancer history, compared to participants with a father, mother, or sibling with cancer history.

3.3 DIFFERENCE BETWEEN RESPECTIVE GROUPS ACCORDING TO METABOLIC STATUS AND BODY MASS INDEX

In order to describe the interaction of both BMI and metabolic status on various anthropometric, BIA and blood-specific parameters, the following groups were identified and compared with each other: (1) non-MetS with normal BMI (NMetS-), (2) MetS with overweight BMI (OWMetS+), (3) non-MetS with overweight BMI (OWMetS-), (4) MetS with obese BMI (OBMetS+), and (5) non-MetS with obese BMI (OBMetS-).

Following BMI calculation, the participants were classified as normal, overweight or obese (Figure 3.5). In the total study population, the majority of participants were categorised as being overweight (n=34, 42.5 %), or obese (n=30, 37.5 %) (Figure 3.5A). In the MetS group, the overweight and obese groups included n=17 participants each (Figure 3.5B). Of the n=46 participants in the non-MetS group, n=16 (34.8 %) presented with normal weight, n=17 (37.0 %) were classified as overweight, and n=13 (28.3 %) were obese (Figure 3.5C).

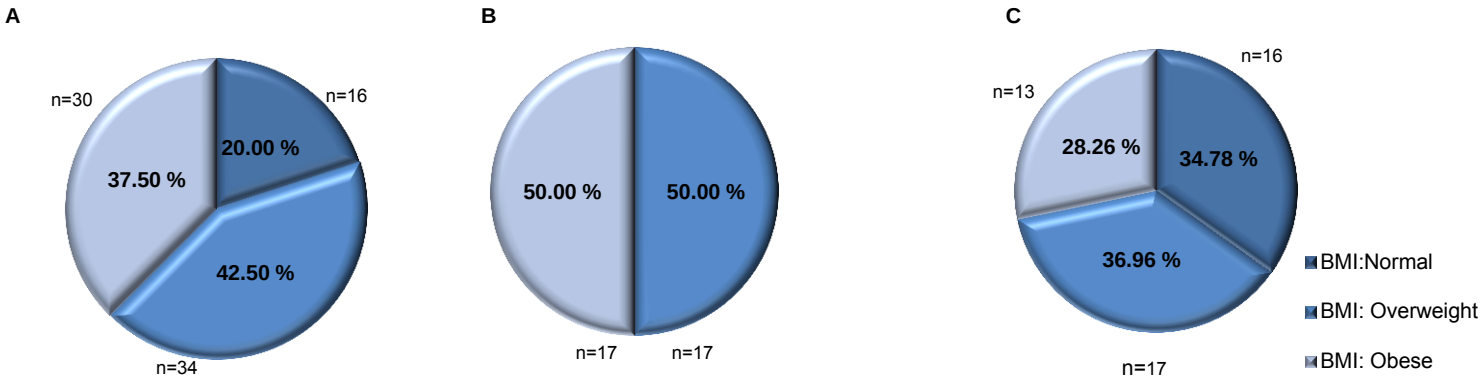

Figure 3.5: The distribution of participants according to their body mass index for (A) the total study population (n=80), (B) the MetS group (n=34), and (C) the non-MetS group (n=46).

3.3.1 Distribution and prevalence of the individual components of the metabolic syndrome

The different components of the MetS (IDF, 2006) are presented in Figure 3.6. Here, WC was significantly higher in all subgroups (OWMetS+, OWMetS-, OBMetS+ and OBMetS-) compared to the NMetS- group ($p<0.001$ for all) (Figure 3.6A). Furthermore, WC measurements for both the OBMetS+ and OBMetS- groups were significantly higher compared to their overweight counterparts (99.7±2.3 *vs* 84.1±0.7 cm for MetS and 95.1±2.2 *vs* 78.0±1.3 cm for non-MetS, $p<0.001$ for both). A statistical trend was also observed between the OWMetS+ and OWMetS- groups (84.1±0.7 *vs* 78.0±1.3 cm, $p=0.06$).

Systolic blood pressure was significantly higher in the OBMetS+ group compared to the OBMetS- group (143.7±4.7 *vs* 123.1±3.5 mmHg, $p<0.05$) (Figure 3.6B). No significant differences were observed for DBP (Figure 3.6C) or blood glucose levels (Figure 3.6D). Both the NMetS- and the OWMetS- groups had higher HDL-c levels compared to the OWMetS+ group ($p<0.05$ for both) (Figure 3.6E). Triglyceride levels were significantly higher in the OWMetS+ group compared to the NMetS- group (2.3±0.3 *vs* 1.1±0.2 mmol/L, $p<0.01$). Additionally, a significant difference was noted between the OWMetS+ and OWMetS- groups (2.3±0.3 *vs* 1.1±0.2 mmol/L, $p<0.01$) (Figure 3.6F).

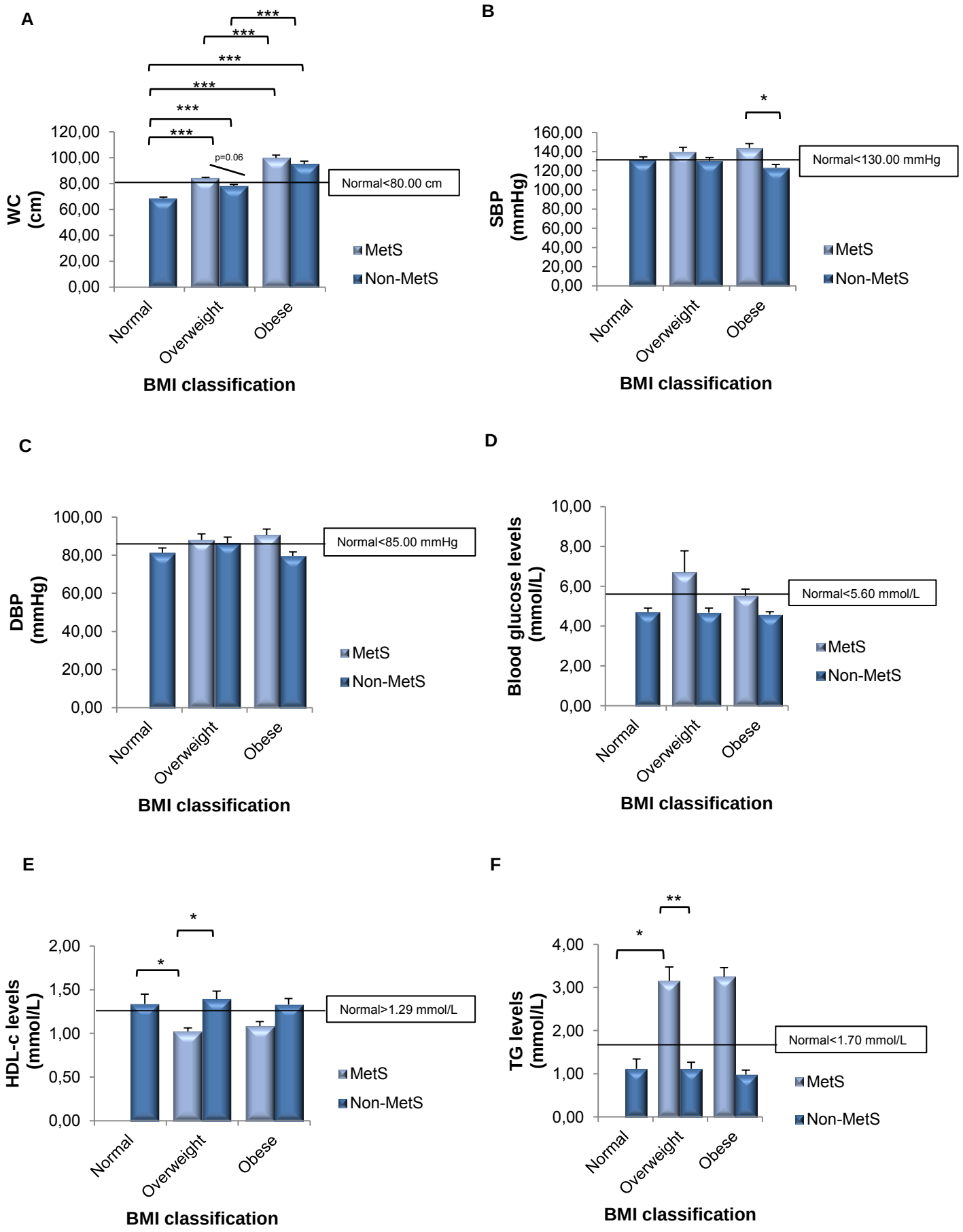

Figure 3.6: MetS risk factors according to IDF criteria for: (A) waist circumference, (B) systolic-, and (C) diastolic blood pressure, (D) blood glucose, (E) high-density lipoprotein cholesterol, and (F) triglycerides. Solid lines represent IDF normal cut-off values (IDF, 2006). * p<0.05, ** p<0.01, *** p<0.001.

3.3.2 Anthropometric parameters

Apart from WC, all other anthropometric indices were grouped and illustrated in Figure 3.7. All groups had significantly higher BMIs compared to the NMetS- group ($p<0.001$ for all except OWMetS-, $p<0.01$ for OWMetS-) (Figure 3.7A). The OBMetS+ and OBMetS- also differed significantly from the OWMetS+ and OWMetS- groups, respectively (37.2±1.0 *vs* 27.9±0.4 kg/m^2 for the MetS groups and 38.5±1.5 *vs* 26.9±0.4 kg/m^2 for the non-MetS groups, $p<0.001$ for both). Similar results were obtained for HC (Figure 3.7B).

The W:H was significantly higher in the OBMetS+ group compared to the NMetS- group ($p<0.01$) (Figure 3.7C). A statistical trend was also observed between the NMetS- and OWMetS+ groups (0.8±0.0 *vs* 0.8±0.0, $p=0.07$). The SAD was significantly higher in all the respective groups ($p<0.001$ for all), except between the NMetS- and OWMetS- group (Figure 3.7D). Furthermore, SAD was significantly higher in the obese compared to the overweight groups (28.1±0.6 *vs* 23.0±0.3 cm for the MetS group and 28.2±0.9 *vs* 21.2±0.4 cm for the non-MetS group, $p<0.001$).

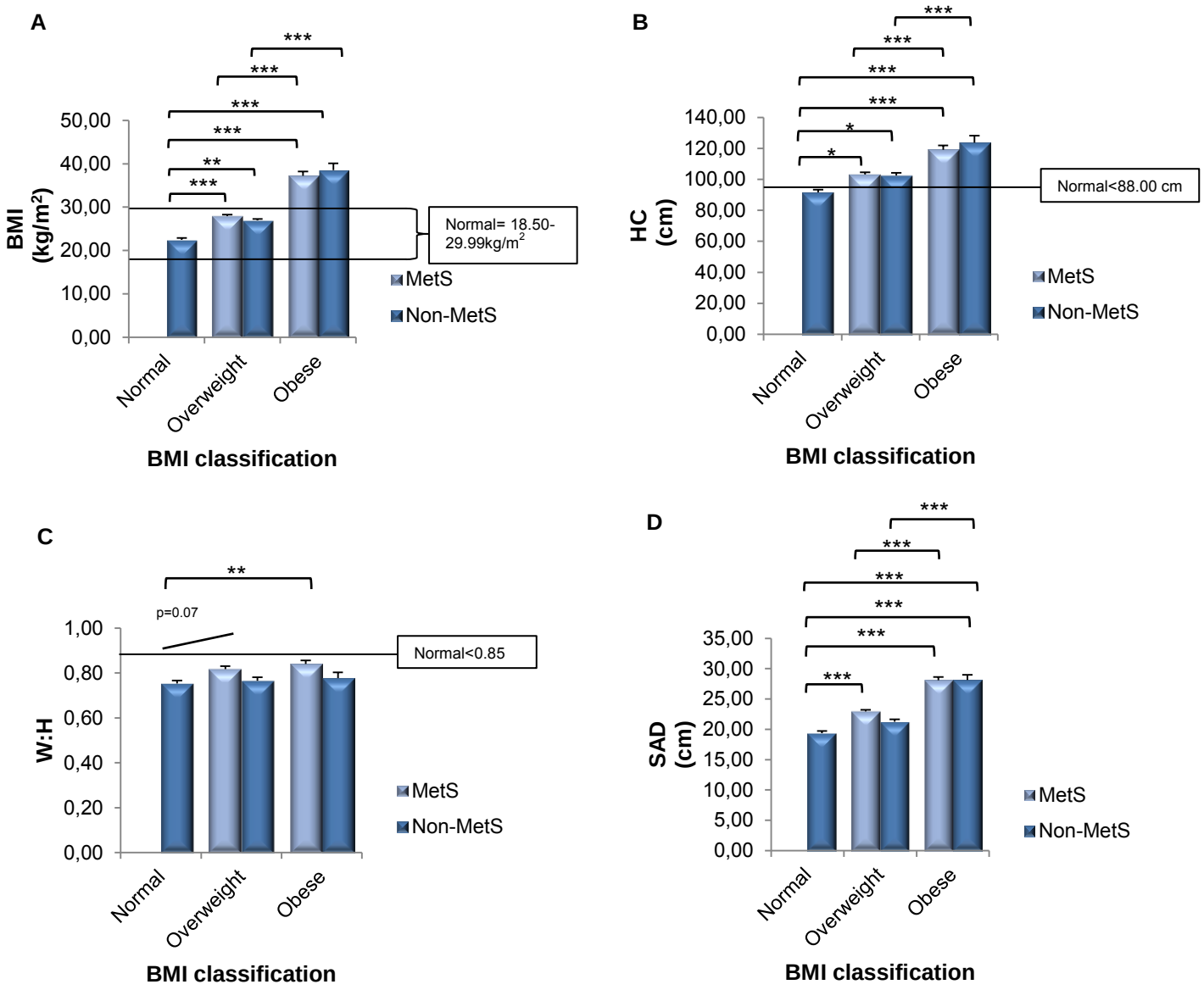

Figure 3.7: Anthropometric indices including, (A) body mass index, (B) hip circumference, (C) waist-to-hip ratio, and (D) sagittal abdominal diameter for all groups, categorised according to metabolic status and body mass index class. Solid lines represent normal WHO cut-off values.
(WHO, 2004a; 2008).
* p<0.05, ** p<0.01, *** p<0.001.

3.3.3 Bioelectrical impedance analysis characteristics

3.3.3.1 Subcutaneous and visceral adipose tissue area

The SAT areas for the OWMetS+, OBMetS+ and OBMetS- groups were significantly greater compared to the NMetS- group (p<0.001 for all). The SAT area of the OBMetS- group was also significantly greater than that of the OWMetS- group (225.0±13.9 *vs* 167.8±10.3 cm^2, p<0.01) (Figure 3.8A).

Similar results were obtained for VAT (p<0.001 for all). Here, a statistical trend was also noted between the OWMetS+ and OWMetS- groups (336.8±6.2 cm^2 *vs* 300.2±13.0, p=0.051) (Figure 3.8B).

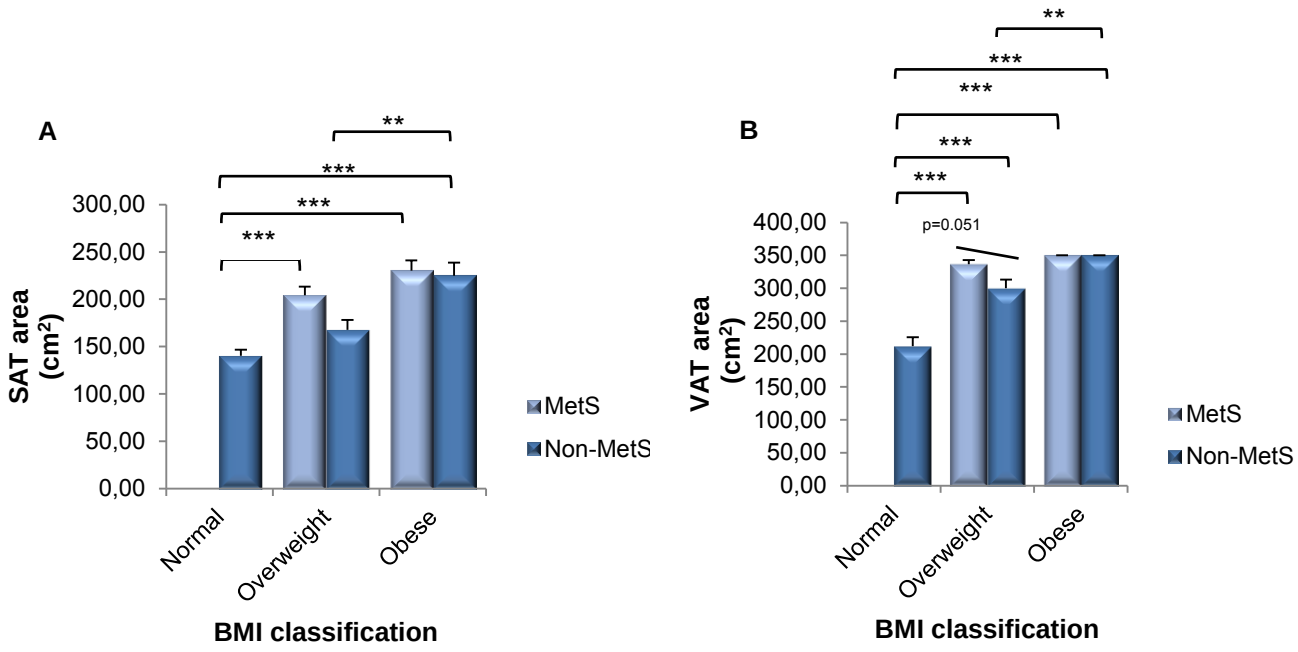

Figure 3.8: Subcutaneous adipose tissue area (A), and visceral adipose tissue area (B), for all groups, categorised according to metabolic status and body mass index class.
* p<0.05, ** p<0.01, *** p<0.001.

3.3.3.2 Percentage subcutaneous and visceral adipose tissue, and VAT:SAT

The SAT % for all subgroups was significantly lower compared to the NMetS- group (p<0.001 for all groups) (Figure 3.9A). All results for VAT % were contrary to that obtained for SAT % (p<0.001 for all groups except OWMetS-, p<0.01) (Figure 3.9B). Significant differences were also evident between the OWMetS+, OWMetS- and their respective obese groups for SAT % and VAT % (68.7±1.4 *vs* 76.1±1.2 % for the MetS groups and 66.9±1.1 *vs* 75.0±1.3 % for the non-MetS groups, p<0.001 for both). The VAT:SAT showed similar results to that obtained for VAT %; however, no significant difference was observed between the NMetS- and OWMetS- (Figure 3.9C).

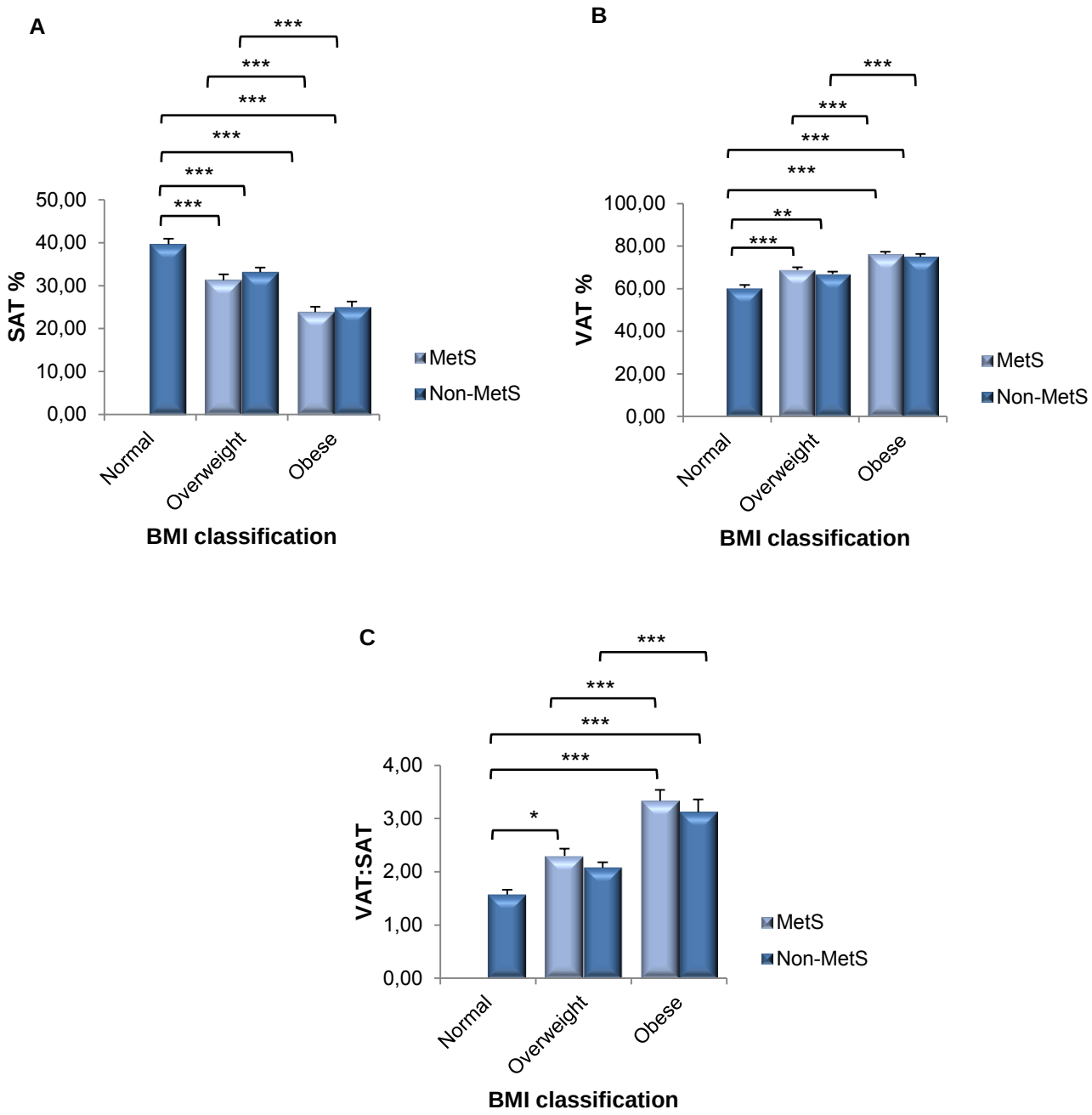

Figure 3.9: Percentage subcutaneous adipose tissue (A), percentage visceral adipose tissue (B), and (C) VAT:SAT for groups, categorised according to metabolic status and body mass index class.
* p<0.05, ** p<0.01, *** p<0.001.

3.3.4 Red blood cell membrane fatty acid profile

The following sections present the results for the Ω-3 index, α-LA and DPA levels, Ω-3:Ω-6, FA metabolism desaturase enzymes, and n-9 SI.

3.3.4.1 The omega-3 index

No significant differences were observed for EPA, DHA, and the Ω-3 index (EPA+DHA) (Figure 3.10).

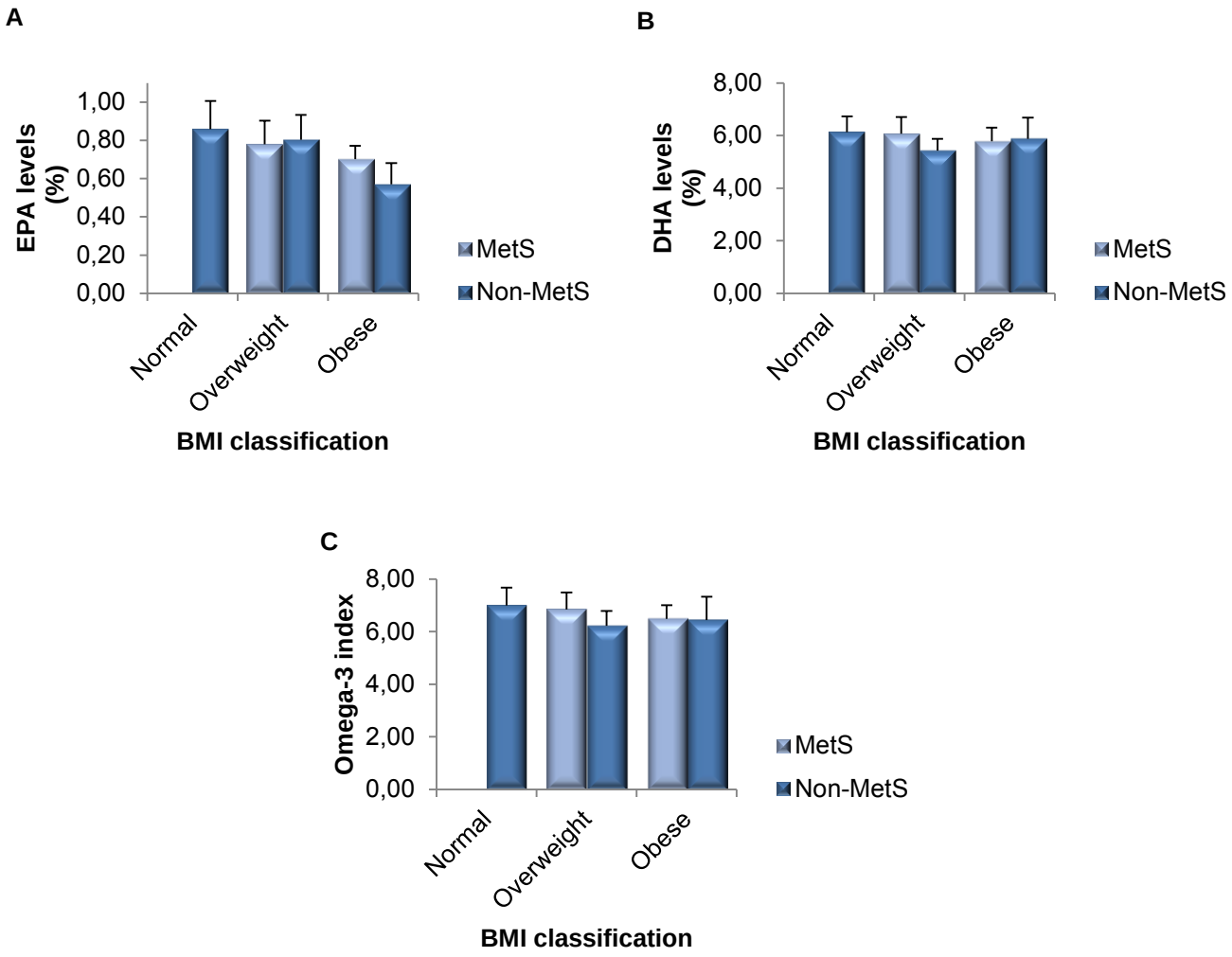

Figure 3.10: Eicosapentaenoic acid (A), docosahexaenoic acid (B), and the omega-3 index (C) for groups, categorised according to metabolic status and body mass index class.
* p<0.05, ** p<0.01, *** p<0.001.

3.3.4.2 Additional omega-3 fatty acids

The results for α-LA and DPA levels did not yield any statistical significant differences (Figure 3.11).

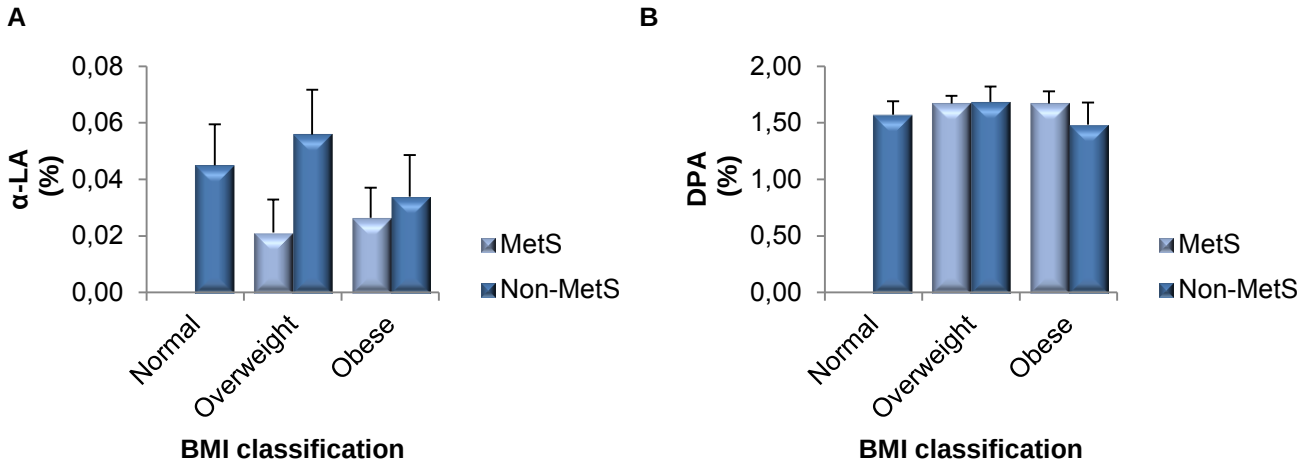

Figure 3.11: Alpha-linoleic acid (A), and docosapentaenoic acid (B) for groups categorised according to metabolic status and body mass index class.
* p<0.05, ** p<0.01, *** p<0.001.

3.3.4.3 The omega-3 to omega-6 ratio

No significant differences were observed for any of the Ω-6 FAs (LA and AA) or the Ω-3:Ω-6 (Figure 3.12).

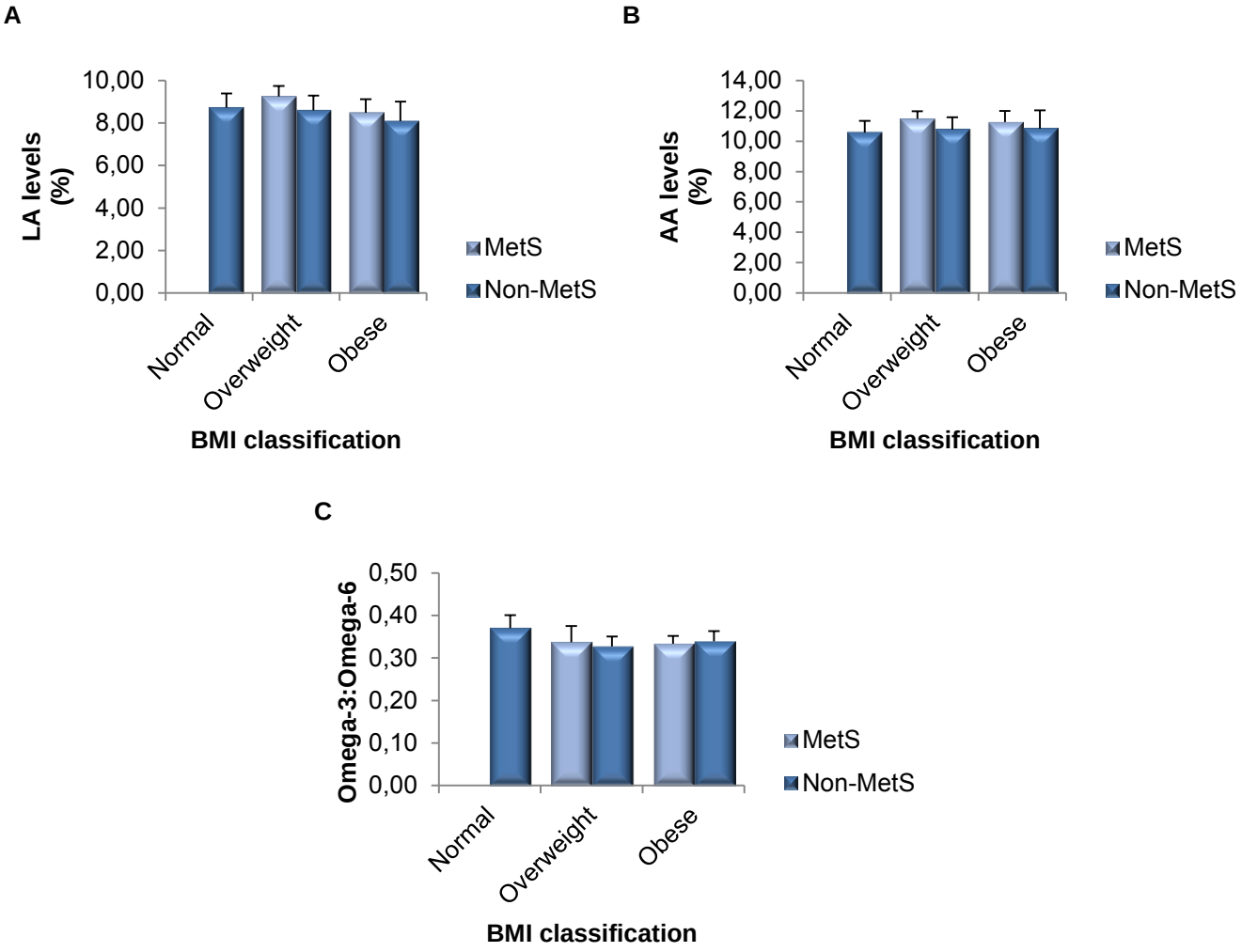

Figure 3.12: Linoleic acid (A), arachidonic acid (B), and the omega-3:omega-6 (C) for groups categorised according to metabolic status and body mass index class.
* p<0.05, ** p<0.01, *** p<0.001.

3.3.4.4 Fatty acid metabolism: delta-9 SCD 1

No significant differences were observed for PA, PLA, and the Δ-9 SCD 1 (PLA/PA) (Figure 3.13).

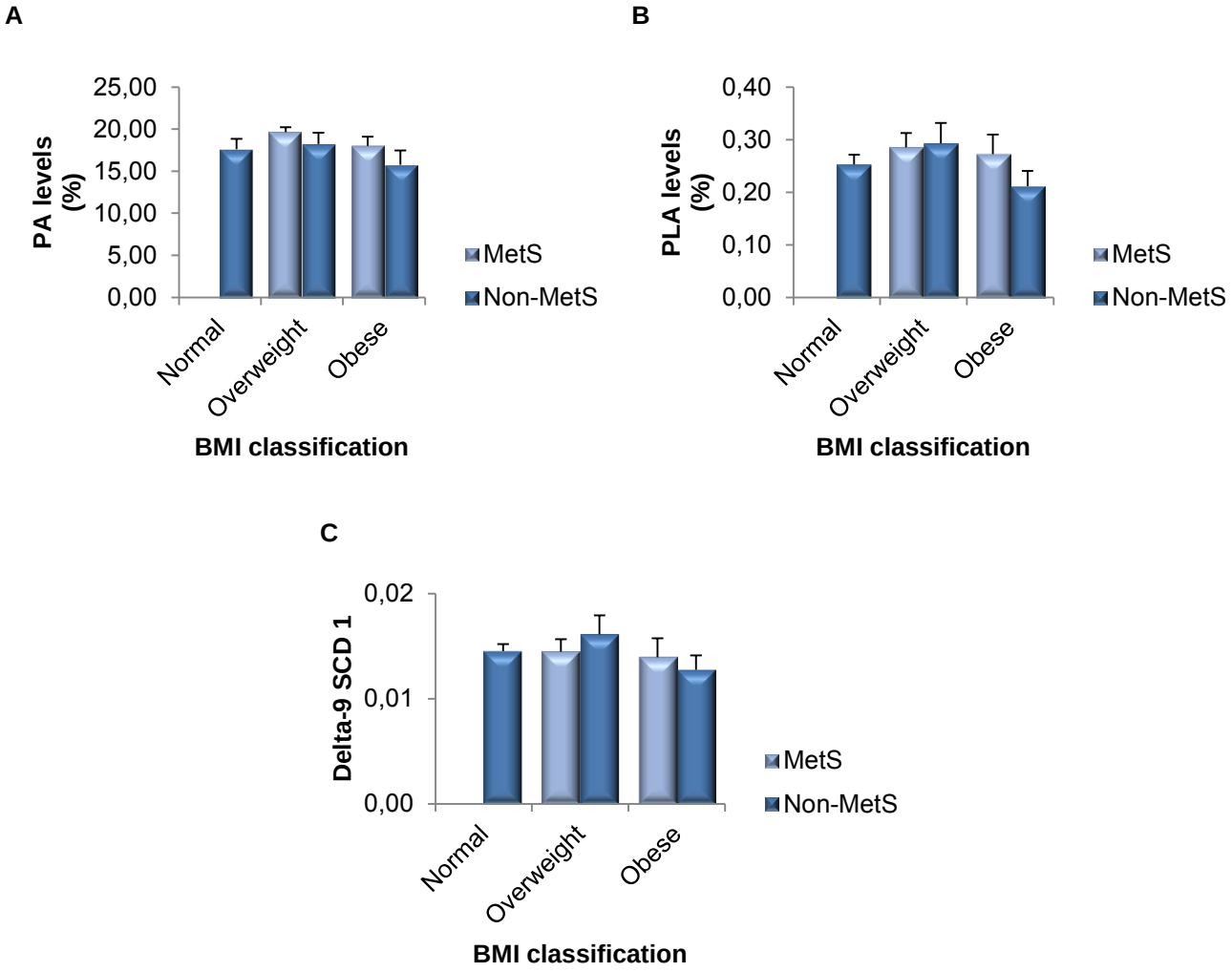

Figure 3.13: Palmitic acid (A), palmitoleic acid (B), and the delta-9 SCD 1 (C) for groups categorised according to metabolic status and body mass index class.
* p<0.05, ** p<0.01, *** p<0.001.

3.3.4.5 Fatty acid metabolism: delta-9 SCD 2 and the n-9 saturation index

Stearic acid, OA and the Δ-9 SCD 2, calculated as OA:SA levels, showed no statistically significant differences (Figure 3.14A-C). The n-9 SI, calculated as SA:OA levels, indicated no statistically significant differences or statistical trends between groups (Figure 3.14D).

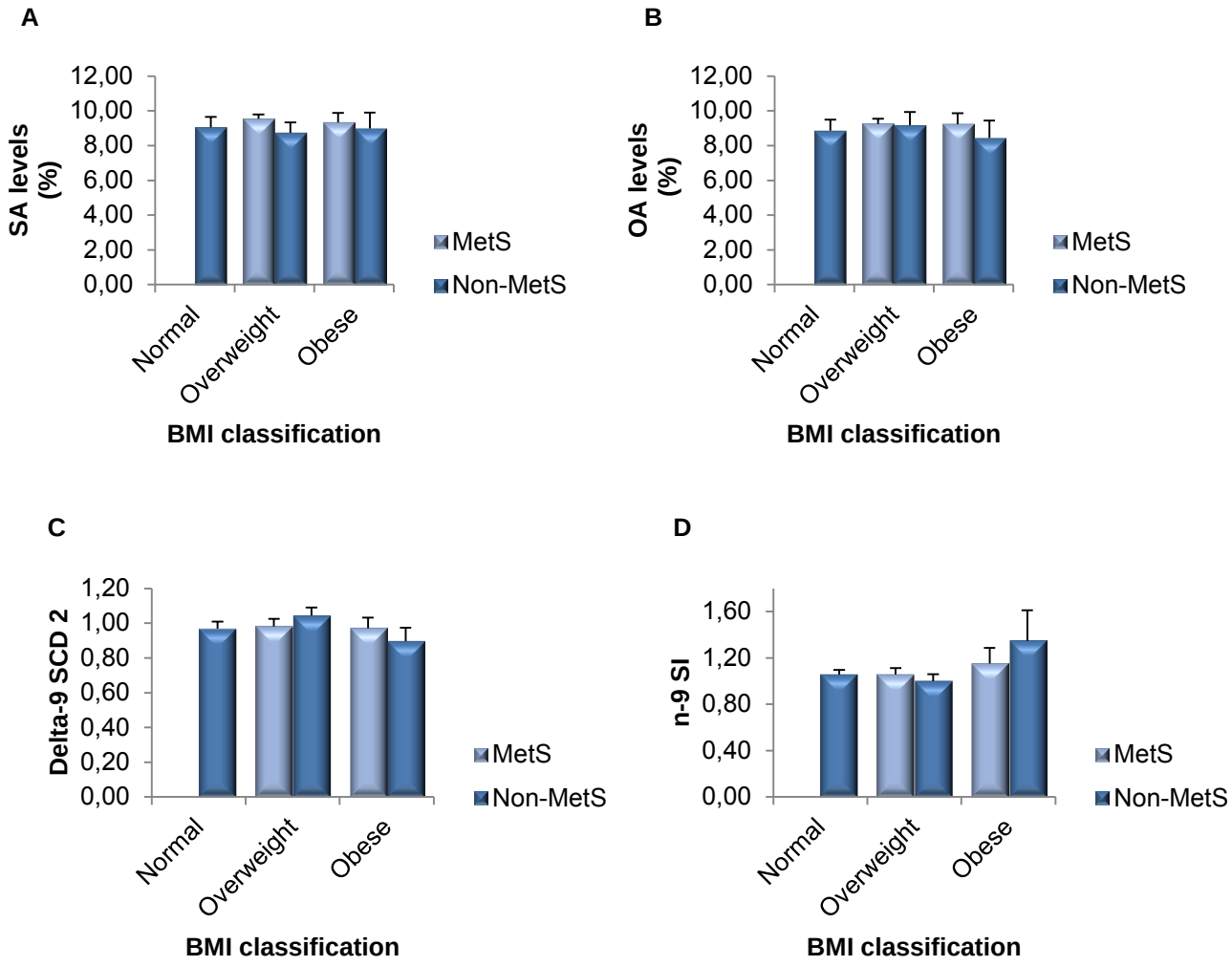

Figure 3.14: Stearic acid (A), oleic acid (B), the delta-9 SCD 2 (C), and the n-9 saturation index (D) for groups categorised according to metabolic status and body mass index class.
* p<0.05, ** p<0.01, *** p<0.001.

3.3.5 Biochemical blood analysis for insulin-like growth factor-1 (IGF-1) and leptin

The IGF-1 concentration of the OBMetS+ group was significantly higher than that of the NMetS- group (121.9±29.9 *vs* 41.0±8.1 ng/mL, p<0.05) (Figure 3.15A). Albeit so, the OBMetS+ presented with IGF-1 concentration two and a half times higher than the OBMetS- women. Although, no statistically significant difference was also noted for leptin concentrations, the OWMetS- group presented with a leptin concentration 1.7 times higher than the OWMetS+ group. Similarly, leptin concentration of the OBMetS+ group was two times higher than the OBMetS- group (Figure 3.15B)

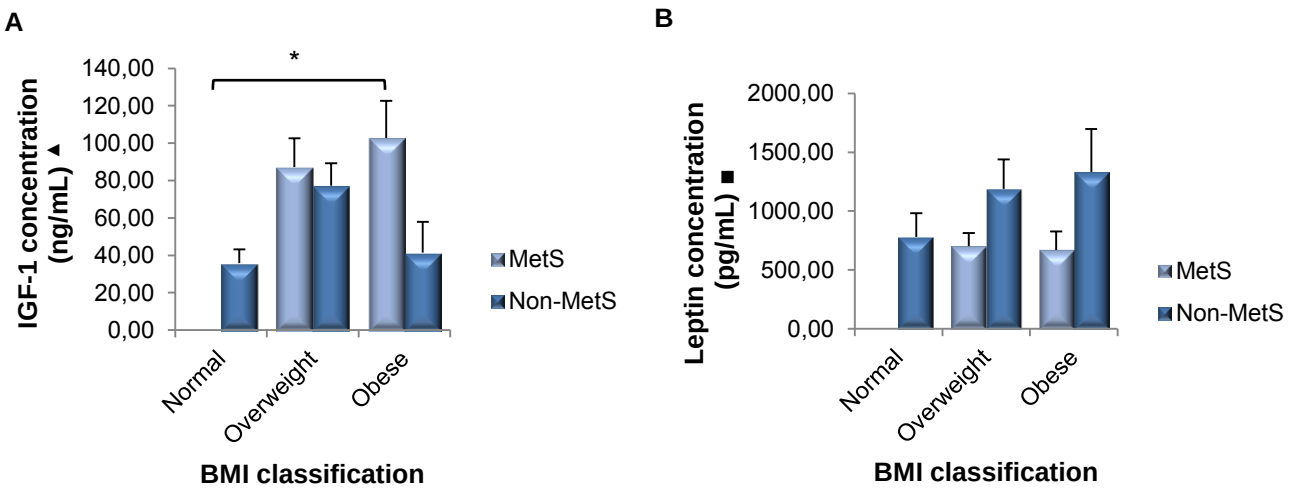

Figure 3.15: Biochemical analysis for (A) insulin-like growth factor-1 and (B) leptin.
* p<0.05, ** p<0.01, *** p<0.001.
▲NMetS- (n=13, 39.4 %), OWMetS- (n=12, 36.4 %), OWMetS+ (n=14, 48.3 %), OBMetS- (n=8, 24.2 %), OBMetS+ (n=15, 51.7 %).
■ NMetS- (n=9, 28.1 %), OWMetS- (n=13, 40.6 %), OWMetS+ (n=13, 46.4 %), OBMetS- (n=10, 31.3 %), OBMetS+ (n=15, 53.6 %).

3.4 THREE-DAY FOOD DIARY

Although a small proportion (n=23, 28.8 %) of the total study participants used in the current study completed the three-day food diaries, a frequency table of the top ten food items for carbohydrate, protein, and fat macronutrient intake are presented in Table 3.5. Potatoes were the most commonly consumed carbohydrate food, followed by breakfast cereals and porridge, and brown- and white bread. Small and equal quantities of crackers, pizzas and pies were also consumed. For most commonly eaten protein-rich foods, the majority of participants consumed full cream milk and cheese, while beef was the most common meat source consumed, followed by chicken and pork. Only a small number of participants consumed lamb and mutton. Sunflower oil, followed by polyunsaturated margarine and hard margarine were the most commonly used fats and oils. Consumption of brick margarine, 50 % polyunsaturated margarine, and salad dressings were similar.

Table 3.5: Frequency table for the three-day food records of carbohydrate, protein, and fat intake.

FREQUENCY	CARBOHYDRATES	PROTEIN	FATS
1	Potatoes	Full cream milk	Sunflower oil
2	Breakfast cereals and porridge	Cheese	Polyunsaturated margarine
3	Brown bread	Beef	Hard margarine
4	White bread	Chicken	Mayonnaise
5	Rice	Pork	Butter
6	Pasta	Low fat and 2 % milk	Olive oil
7	Muffins, cookies, cakes and puddings	Eggs	Butro
8	Crackers	Fish	Brick margarine
9	Pizza	Lamb and mutton	50 % polyunsaturated margarine
10	Pies	Full fat yoghurt	Salad dressing

From all the carbohydrates, protein and fats listed in Table 3.5, the top ten food products consumed were also determined (Figure 3.16). Full cream milk was the most common food

product consumed by participants. Similar quantities of cheese and sunflower oil were consumed, as well as equal quantities of pork, and low fat and two percent milk.

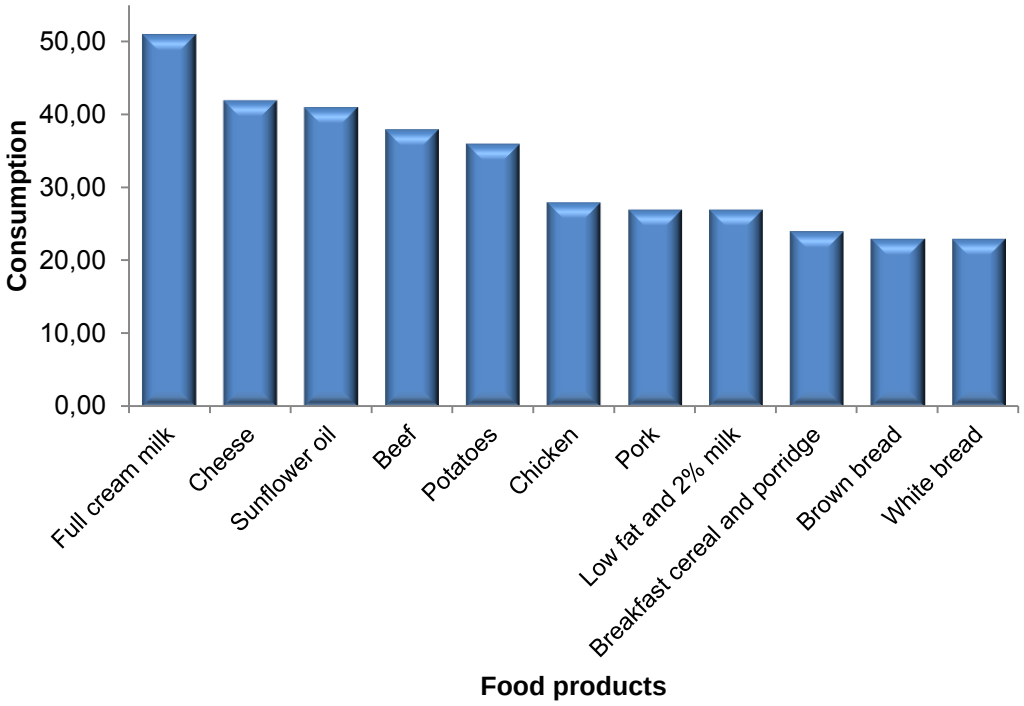

Figure 3.16: The frequency of the top ten food products consumed by the study participants.

3.5 CORRELATION ANALYSES

Correlations were performed in order to establish the possible associations between selected physiologically-relevant parameters. The following correlation coefficient values were considered: (i) r-values greater than 0.70 were regarded very strong, (ii) r-values between 0.40 and 0.69 as strong, (iii) r-values between 0.30 and 0.39 as moderate, and (iv) r-values between 0.20 and 0.29 as weak. R-values less than 0.19 indicated no relationship (Scally, 2014). For all correlation purposes, the NMetS- groups are indicated in the figures labelled A and D (G, J and M), the OWMetS+ and OWMetS- groups in figures B and E (H, K and N), and the OBMetS+ and OBMetS- groups in figures C and F (I, L and O).

3.5.1 Body mass index correlations

Body mass index positively correlated with the VAT:SAT for all groups, except the OWMetS+ group (Figure 3.17A-C). Significant strong correlations were only observed in the obese groups (r=0.67, p=0.00 for OBMetS+ and r=0.61, p=0.00 for OBMetS-) (Figure 3.17C). Similar results were observed between BMI and VAT % (Figure 3.17D-F). Here, the OBMetS+ and the OBMetS- correlations were also significant (r=0.64, p=0.01 for OBMetS+ and r=0.57; p=0.04 for

OBMetS-) (Figure 3.17F). Although the correlation between BMI and SAT % showed opposing results (Figure 3.17G-I), the OBMetS+ and OBMetS- groups' correlations were also significant (r=-0.64, p=0.01 for OBMetS+ and r=-0.57, p=0.04 for OBMetS-), with similar correlation coefficients and p-values to that observed for the correlation between BMI and VAT %.

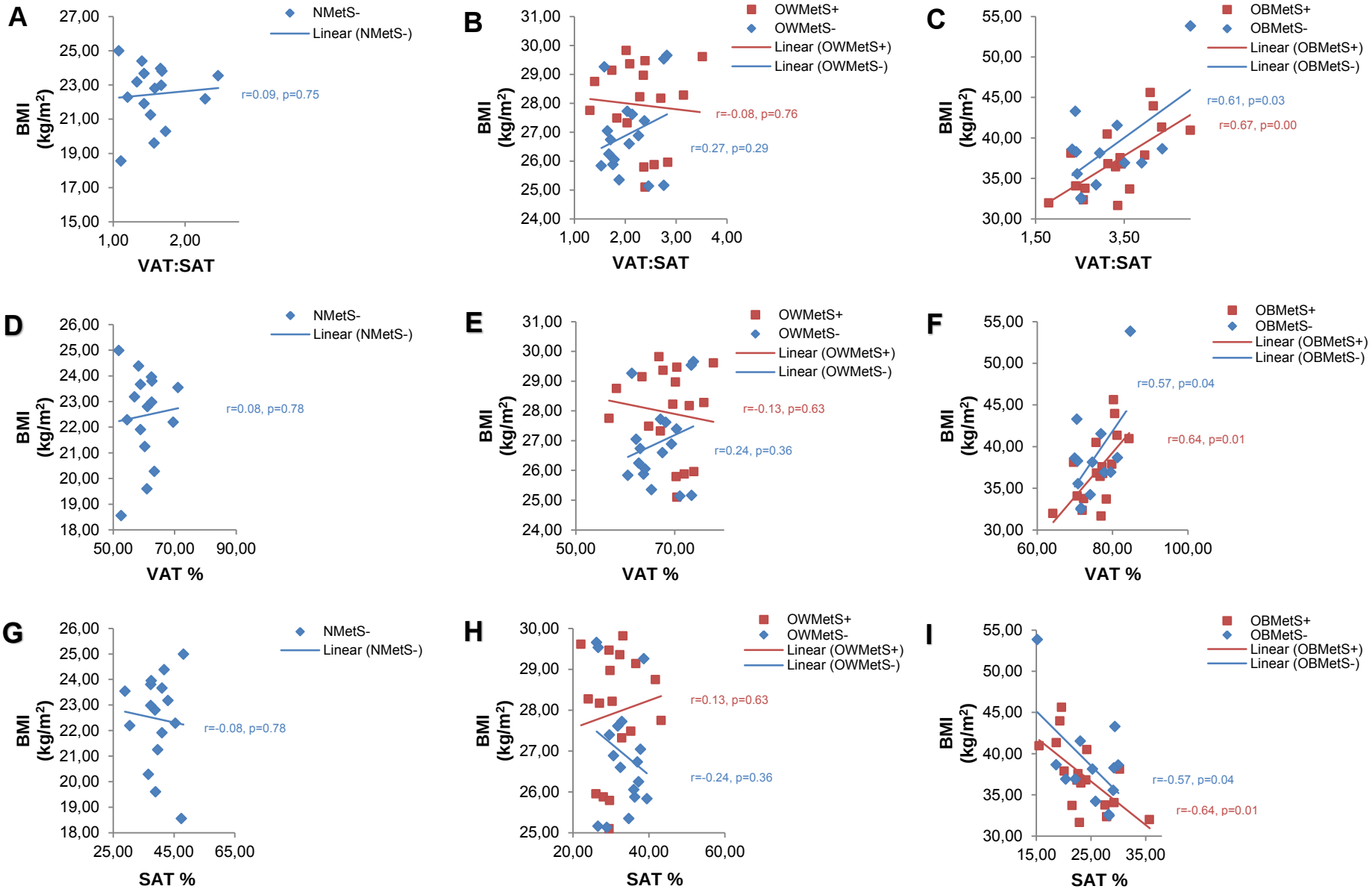

Figure 3.17: Associations between body mass index and VAT:SAT, body mass index and percentage visceral adipose tissue, and body mass index and subcutaneous adipose tissue in the NMetS- (A, D, G), OWMetS+ and OWMetS- (B, E, H), and OBMetS+ and OBMetS- (C, F, I) groups.

3.5.2 Systolic blood pressure correlations

Systolic blood pressure was correlated with EPA (Figure 3.18A-C), DPA (Figure 3.18D-F), DHA (Figure 3.18G-I), Ω-3 index (Figure 3.18J-L) and Ω-3:Ω-6 (Figure 3.18M-O), with most of the correlations either showing a weak relationship or no association. Moderate to strong positive associations were evident when SBP was correlated with EPA levels in all groups, except for the NMetS- and OBMetS- groups (Figure 3.18A and 3.18C). When SBP was correlated with DPA, weak and strong negative correlations were evident in the OWMetS- and OBMetS- groups, respectively (Figure 3.18E and 3.18F). For SBP and DHA, a weak negative correlation was also evident in the OBMetS- group, while a strong positive association was observed for the OWMetS+ group (Figure 3.18H and 3.18I). Similar associations were evident when SBP was correlated with the Ω-3 index, while strong positive trends were observed between SBP and EPA ($r=0.46$, $p=0.06$) (Figure 3.18B), and SBP and DHA ($r=0.45$, $p=0.07$) (Figure 3.18H). A strong positive trend was also evident for SBP and the Ω-3 index ($r=0.46$, $p=0.06$) (Figure 3.18K), while a significant positive correlation was observed for SBP and the Ω-3:Ω-6 ($r=0.49$, $p=0.046$) in the OWMetS+ group (Figure 3.18N).

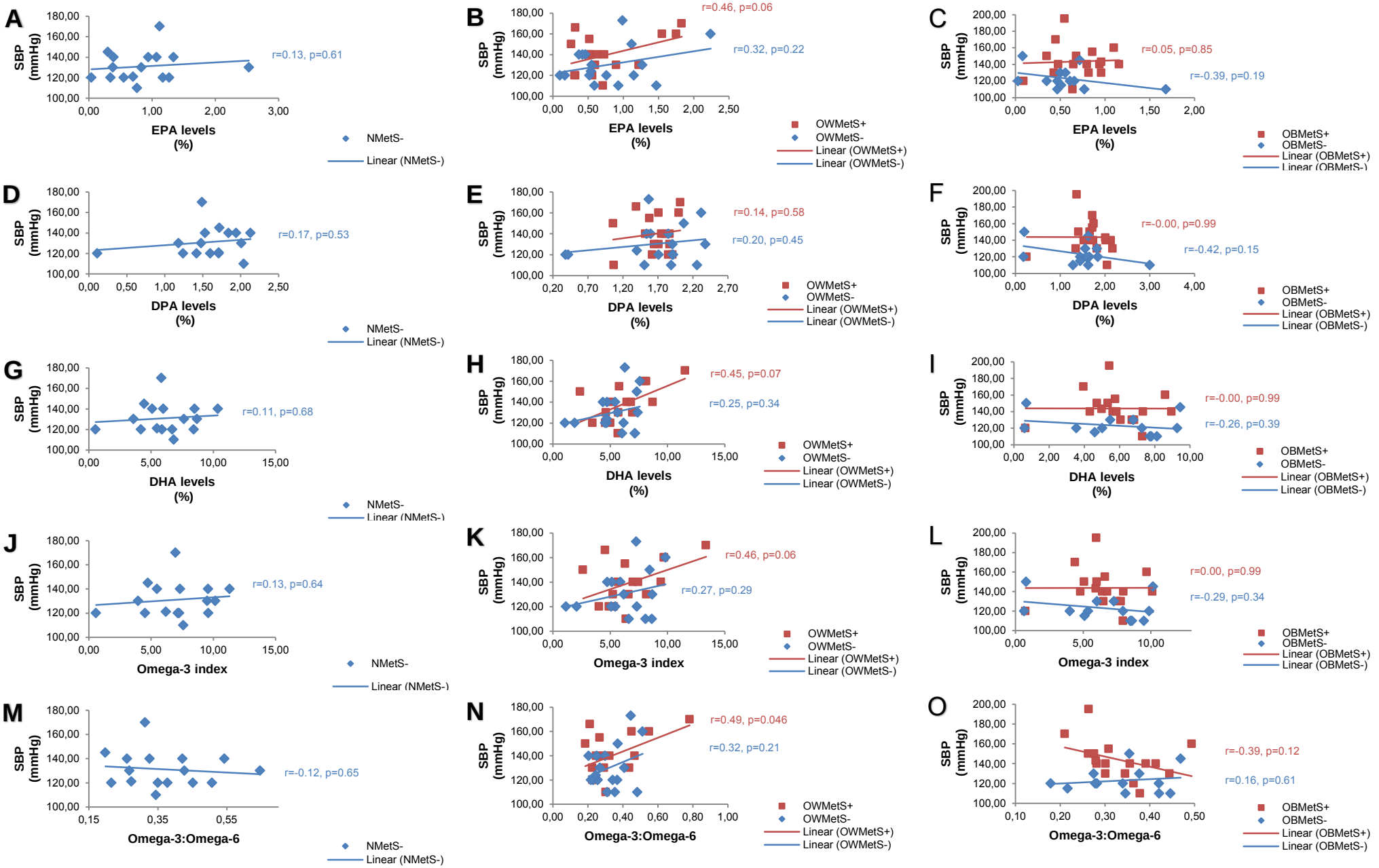

Figure 3.18: Correlations between systolic blood pressure and eicosapentaenoic, docosapentaenoic and docosahexaenoic acid levels, the omega-3 index, and the omega-3:omega-6 in the NMetS- (A, D, G, J, M), OWMetS+ and OWMetS- (B, E, H, K, N), and OBMetS+ and OBMetS- (C, F, I, L, O) groups.

3.5.3 Subcutaneous adipose tissue area correlations

Subcutaneous adipose tissue area was positively correlated with the Ω-3 index (Figure 3.19A-C) and Ω-3:Ω-6 (Figure 3.19D-F) for all groups, except the NMetS- group. Significant, strong positive correlations between SAT area and the Ω-3 index were evident in the OWMetS+ group (r=0.51, p=0.04) (Figure 3.19B), and the OBMetS- group (r=0.56, p=0.04) (Figure 3.19C). Correlations between the SAT area and the Ω-3:Ω-6 indicated statistically significant, strong positive correlations in the OWMetS+ (r=0.54, p=0.02) OBMetS+ groups (r=0.51, p=0.04).

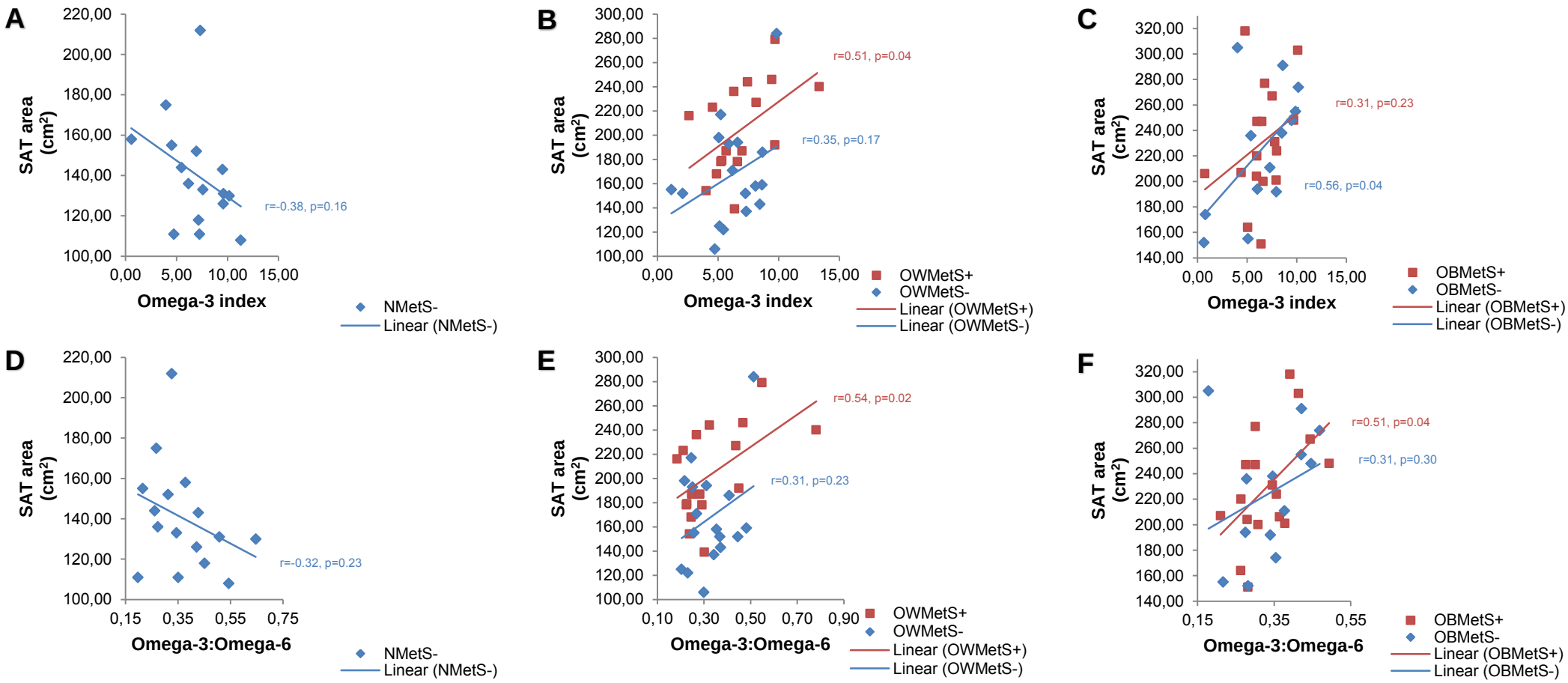

Figure 3.19: Relationship between the subcutaneous adipose tissue area and the omega-3 index, and subcutaneous adipose tissue and the omega-3:omega-6 in the NMetS- (A, D), OWMetS+ and OWMetS- (B, E), and OBMetS+ and OBMetS- (C, F) group.

CHAPTER 4: DISCUSSION

4.1 INTRODUCTION

The present study aimed to describe the prevalence of the MetS in a female farm worker population. Furthermore, the associations between the components of the MetS and specific individual FAs and FA ratios, anthropometric measures, dietary intake, and possible cancer risk were also explored. This chapter will focus on interpreting the results found in the current study by using relevant literature.

4.2 PREVALENCE OF THE METABOLIC SYNDROME

It was reported in a few studies reported on the prevalence of the MetS on the African continent. Several factors are at play that could help explain the lack of information on prevalence studies in SSA, including the use of different definitions to identify the MetS. Studies conducted in Europe, China, Iran, and Mexico have shown the MetS prevalence to range between 21.1 to 33.8 % (Alkerwi *et al.*, 2011; Esteghamati *et al.*, 2011; Chiu *et al.*, 2012; García-Jiménez *et al.*, 2015). A study in South Africa reported that 42.1 % of the population presented with the MetS using the JIS definition (Crowther & Norris, 2012). Two studies, one in Botswana and the other in Nigeria, found the MetS prevalence to differ significantly when using the ATP III criteria. Gyakobo *et al*. (2012) found the MetS prevalence in Ghana to be as high as 35.9 %, much higher than the MetS prevalence of 12.1 % reported in Nigeria (Adegoke *et al.*, 2010). It was reported by a South African study that 25.0 % of women from Kwa-Zulu Natal presented with the MetS with IDF criteria among (Motala *et al.*, 2011), while Erasmus *et al.* (2012) described a higher MetS prevalence of 67.8 % among women in the Western Cape Province using the IDF definition. A bit closer to home, a more recent Cape Town study by Peer *et al.* (2015), reported a much lower overall MetS prevalence of 30.7 % (43.5 % for women and 16.5 % for men), when using the JIS definition. Our population, although only females, had a prevalence of 42.5 % when IDF criteria was used (Figure 3.1), which is still considered high compared to other South African studies.

Several inconsistencies between these studies and the present study may contribute to the different findings, one of which includes the sample size. Both Motala *et al.* (2011) and Erasmus *et al.* (2012) included much larger sample sizes (n=947 and n=642, respectively) compared to the sample size of the current study (n=80). The present study included Caucasian, Coloured, and Black women, whereas Motala *et al.* (2011) and Erasmus *et al.* (2012) only included Black and Coloured participants, respectively. Lifestyle- and genetic factors may also have influenced the difference in prevalence. Poor dietary choices, including high intake of salt, processed foods,

and saturated and *trans*-fat, all contribute to the increased prevalence of the individual MetS components, which ultimately increase the risk of developing the MetS (Jeon *et al.*, 2011; Peer *et al.*, 2013; Helelo *et al.*, 2014). Smoking, alcohol consumption, and physical inactivity are also considered major risk factors for the components of the MetS (Helelo *et al.*, 2014). Dietary intake of the women of the current study indicated a generally unhealthy diet (Table 3.5), with most women being current drinkers (Appendix I.1) who participated in moderate-intensity work and recreational activities (Appendix I.2), the majority of which did not smoke (Appendix I.1). It is therefore likely that diet, alcohol consumption, and physical activity may have contributed to the high prevalence of the MetS in our population.

Age is also considered a confounding factor, since the females' mean age in the present study was 37.2 years, while the mean age in the studies by Motala *et al.* (2011) and Erasmus *et al.* (2012) ranged between 46.5 years and 50.9 years, respectively. Ageing is associated with an increased prevalence of the individual MetS components and an increased risk of developing the MetS (Alkerwi *et al.*, 2011; Camhi *et al.*, 2011; Peer *et al.*, 2013; Helelo *et al.*, 2014; Rochlani *et al.*, 2015). This was also evident in the current study, since the participants in the MetS group (mean age 40.2 years) were significantly older than their non-MetS (mean age 35.0 years) counterparts (Table 3.1).

The effect of gender may also contribute to the discrepancies between these studies, since findings suggest that the prevalence of the MetS is higher in women (Garrido *et al.*, 2009; Motala *et al.*, 2011, Erasmus *et al.*, 2012; Gyakobo *et al.*, 2012; Peer *et al.*, 2015). For example, Peer *et al.* (2015) reported a MetS prevalence of 43.5 % among women, while only 16.5 % of men presented with the MetS. It is also likely that gender disparities in lipid- and glucose metabolism could have accounted for the variation in the prevalence of the individual components of the MetS (Hoebel *et al.*, 2013; Rochlani *et al.*, 2015).

The studies mentioned thus far also made use of different MetS definitions. Some studies actually tried to establish the prevalence of the MetS by comparing different definitions with one another to determine which definition would yield a higher MetS prevalence. Alkerwi *et al.* (2011) compared the MetS prevalence in women using the JIS, IDF, and ATP III definitions and found almost similar results (JIS: 20.4 %, IDF: 19.3 % and ATP III: 18.5 %). However, when the IDF definition was compared to that of the ATP III, Gyakobo *et al.* (2012) reported a two times higher MetS prevalence according to the IDF definition (35.9 % *vs* 15.0 %). Reasons for this discrepancy include the lower cut-off values for WC (female WC≥80 cm for IDF and ≥88 cm for ATPII) and FBG (≥5.6 mmol/L for IDF and ≥6.1 mmol/L for ATP III) used in the IDF definition.

4.3 DISTRIBUTION AND PREVALENCE OF THE METABOLIC SYNDROME RISK FACTORS

Although we report a relatively high prevalence of the MetS, the specific combination of risk factors, or even the prevalence of an individual risk factor, are extremely important, since these risk factors can individually, or collectively increase the risk for the development of CVD, T2DM, and lifestyle-associated cancers (Alberti *et al.*, 2009; Klug *et al.*, 2012; Crowther & Norris, 2012; Erasmus *et al.*, 2012; Roswall *et al.*, 2014; Almquist *et al.*, 2015). For the Mets group, the majority of participants presented with three risk factors (58.8 %). However, what is somewhat concerning is that 20.6 % of the participants in the MetS group presented with four and five risk factors respectively, while only 13.0 % of participants in the non-MetS group presented with no MetS risk factors and were therefore not at risk of developing the MetS. Similar to findings from Strand *et al*. (2015), our results showed that in the non-MetS group, two risk factors were most prevalent (54.3 %) (Figure 3.2).

The prevalence of each of the individual MetS risk factors in the present study also differed between the two groups. Abdominal obesity was the most prevalent individual risk factor in the MetS group, which is expected since WC is the determining factor for MetS diagnosis according to the IDF. This was followed by hypertension (82.4 %), and low HDL-c levels (76.5 %) (Figure 3.3). Our finding of the clustering of WC, hypertension, and low HDL-c levels is in agreement with that reported by Gyakobo *et al.* (2012). Here, the IDF definition was also used and the same three risk factors were shown to be largely responsible for the MetS prevalence in a rural population from Ghana.

For the non-MetS group of the present study, hypertension was the most common individual MetS risk factor (47.8 %), followed by abdominal obesity (39.1 %), and low HDL-c levels (34.8 %). Contrasting results have been reported for the least, and most prevalent individual MetS risk factors, since earlier studies by Garrido *et al.* (2009) (using the ATP III definition) and Motala *et al.* (2011) (using the JIS definition) found low HDL-c levels to be the most prevalent MetS risk factor. However, more recent studies found increased WC to be more prevalent (Hoebel *et al.,* 2011; Gyakobo *et al.,* 2012; Peer *et al.*, 2015; Salonen *et al.*, 2015; Strand *et al.,* 2015). Although the IDF definition do not routinely use the BMI to assess obesity, studies have indicated that obesity is a major contributor to dyslipidaemia (de Boer *et al.*, 2011; Evans *et al.,* 2011), which was also a common risk factor in the women of the current study. Here, the mean BMIs for the MetS and non-MetS groups were 32.6 kg/m^2 and 28.7 kg/m^2 respectively (Table 3.1), which is indicative of an overweight and obese population, and studies have shown that under these conditions, lipolysis is increased and HDL-c levels are reduced (Boden, 2009;

Mendonça *et al*., 2015). Furthermore, ethnicity may also influence lipid profiles, since a study by Evans *et al.* (2011) observed that Black women exhibited significantly lower HDL-c levels compared to Caucasians, possibly due to the higher prevalence of obesity among Black compared to Caucasian women. The current study included Black, Coloured, and Caucasian women, and inclusion of the different ethnic groups may have influenced the prevalence of specific MetS risk factors.

A recent investigation by Strand *et al.* (2015) found that hypertension was the second most prevalent individual risk factor, which is similar to our findings for the MetS group. Ageing is associated with arterial thickening and increased arterial resistance, which contributes to hypertension (Helelo *et al.*, 2014). The MetS group (mean age 40.2 years) of the current study was significantly older than their non-MetS (mean age 35.0 years for non-MetS) counterparts, which may also explain the prevalence of hypertension in this population, specifically in the MetS group (Table 3.1).

Several lifestyle factors may also contribute to the development of hypertension, including diet, tobacco use, alcohol intake, and physical inactivity (Jennings *et al.*, 2009; Jeon *et al.*, 2011; Peer *et al.*, 2013, Helelo *et al.*, 2014; Kaur, 2014). Even though the participants in the current study were not grouped according to number of cigarettes smoked, or amount of alcohol units consumed, fewer women in the MetS group were current smokers and drinkers compared to the non-MetS group (35.3 *vs* 43.5 % for current smokers, and 55.9 *vs* 71.7 % for current drinkers) (Appendix I.1). As a result, it is unlikely that smoking and alcohol consumption could potentially have contributed to the high prevalence of hypertension in this population.

Concerning physical activity, 17.7 % of MetS and 20.0 % of the non-MetS participants did vigorous-intensity work, while 5.9 % of MetS and 10.9 % of the non-MetS participants did vigorous-intensity recreational activities (Appendix I.2). Fewer participants in the MetS group (50.0 % *vs* 55.6 %) participated in moderate-intensity work, whereas more participants in the MetS group (76.5 % *vs* 69.6 %) participated in moderate-intensity recreational activities. It is therefore possible that physical activity or lack thereof in the MetS group could have potentially contributed to the high prevalence of the individual components of the MetS in this population. Although stress and levels of urbanisation were not assessed in the current study, these factors are known to contribute to hypertension (Jennings *et al.*, 2009; Jeon *et al.*, 2011; Peer *et al.*, 2013, Helelo *et al.*, 2014; Kaur, 2014), and might be worth investigating in the future.

Studies have indicated that abdominal obesity plays a significant role in hypertension, since obesity is associated with microvascular dysfunction and abnormal vascular tone, which

increases vasoconstriction and leads to hypertension (de Boer *et al.*, 2011; Peer *et al.*, 2013, Helelo *et al.*, 2014; Kaur, 2014). Several measures used to assess abdominal obesity were significantly higher in the MetS group of the current study, indicating that obesity may have played a role in the prevalence of hypertension in this population (Table 3.1). Another component of the MetS, glucose intolerance/IR, have also been proposed to contribute to hypertension, although the exact mechanism is still unclear (Jennings *et al.*, 2009; Kirk & Klein, 2009; Horita *et al.*, 2011; Sossa *et al.*, 2012; Kaur, 2014; Soleimani, 2015). Although one such mechanism proposes that IR attenuates the vasodilatory effects of insulin, and this contributes to hypertension (Kirk & Klein, 2009; Horita *et al.*, 2011), not all are in consensus. An alternative mechanism may involve the RAAS system. Here, hyperglycaemia and -insulinaemia are proposed to cause increased angiotensin II expression that activates RAAS, which result in increased BP *via* increased sodium resorption and cardiac output, and ultimately vasoconstriction (Horita *et al.*, 2011; O'Neill & O'Driscoll, 2015; Soleimani, 2015). Glucose and insulin levels were both significantly higher in the MetS group of the current study (Table 3.1), suggesting that glucose intolerance/IR may have played a role in the prevalence of hypertension.

4.4 DESCRIPTIVE PROFILE OF THE STUDY POPULATION ACCORDING TO METABOLIC STATUS AND BODY MASS INDEX

Although BMI is not routinely used by the IDF to assess adiposity, a large body of scientific evidence indicate that an increased BMI is associated with a worsening metabolic profile, since BMI was positively correlated with hypertension, IR, dyslipidaemia, and an increased risk of the MetS and CVD (Wildman *et al.*, 2008; Jeon *et al.*, 2011; Peer *et al.*, 2013; Abu-Farha *et al.*, 2014; Helelo *et al.*, 2014). Various studies indicated that individuals with the MetS had significantly higher BMIs, which suggest a definite link between the MetS and obesity (Nigam *et al.*, 2009; Bremer *et al.*; 2011; Esteghamati *et al.*, 2011; Atanassova *et al.*, 2014). It is suggested that obesity and its associated metabolic alterations contributes to alterations in glucose homeostasis, increased hepatic glycogenesis, and IR that leads to a greater risk of other co-morbidities (Aballay *et al.*, 2013). These studies, as well as several others established that obesity is a dominant risk factor for the MetS, and it is exactly for that reason that BMI was also used in the current study to try to determine the effect of the metabolic status together with BMI on different physiologically relevant parameters.

4.4.1 Body mass index classification

Studies found that approximately 2.1 billion adults were either overweight or obese, with an increasing prevalence of female obesity in low- to middle-income countries (Garrido *et al.*, 2009;

Alkerwi *et al.*, 2011; Crowther & Norris, 2012; Erasmus *et al.*, 2012; Peer *et al.*, 2013; Ng *et al.*, 2014; Senekal *et al.*, 2015). In South Africa, 69.3 % of women were overweight, while 42.0 % were obese (Ng *et al.*, 2014). This trend of more individuals being overweight was also observed in the current study population, with only 20.0 % of the women showing normal body weight (Figure 3.5A). The MetS group of the present study included equal numbers of overweight (n=17, 50.0 %) and obese (n=17, 50.0 %) participants, while the non-MetS group included normal weight (n=16, 34.8 %), overweight (n=17, 37.0 %), and obese (n=13, 28.3 %) women (Figure 3.5B & 3.5C). This could have accounted for the significantly higher mean BMI evident in the MetS group.

The current study also observed that when BMI was used to categorise individuals, the BMIs in all the respective groups were significantly higher compared to the NMetS- group, and the BMI of both obese groups were significantly higher than their overweight counterparts (Figure 3.7A). These findings differ from that found by Senekal *et al.* (2015), who reported that obesity was more common than being overweight (55.0 % *vs* 27.0 %), in women in the Western Cape. The present study and the study by Senekal *et al.* (2015) included participants with a low socio-economic status; however, the study by Senekal *et al.* (2015) included women from urban and rural areas, and level of urbanisation have been shown to be positively correlated with the prevalence of obesity.

Urbanisation is often associated with lifestyle changes, including tobacco use, unhealthy diet, excessive alcohol consumption, and physical inactivity, and these factors contribute to the development of obesity (Erasmus *et al.*, 2012; Hoebel *et al.*, 2013). The majority of women in both the MetS (50.0 %) and non-MetS (45.7 %) groups in the current study were non-smokers, while fewer women in the MetS group (35.3 %) were current smokers than in the non-MetS group (43.5 %) (Appendix I.1). This makes it unlikely that smoking contributed to obesity in this population. Dietary intake in the current study did not reveal a healthy diet plan, since large quantities of SFA, red meat, and refined carbohydrates were consumed, while intake of fruit, vegetables and dietary fibre were low (Table 3.5), and this may have contributed to the prevalence of overweight and obesity in these women. Additionally, although the majority of participants in the current study were current alcohol consumers, current drinking was less common among the MetS (55.9 %) than non-MetS (71.7 %) women (Appendix I.1). Alcohol intake is known to increase obesity, due to increased energy intake (Hoebel *et al.*, 2011; Strand *et al.*, 2015), and this may have played a role in the current study. Physical inactivity could also have contributed to the prevalence of obesity in this population, since fewer participants in the MetS group (50.0 % for the MetS and 55.6 % for the non-MetS) participated in moderate-intensity work (Appendix I.2), compared to a greater proportion participating in moderate-

intensity recreational activities (76.5 % *vs* 69.6 %). Only a small proportion of women in both the MetS (5.9 %) and non-MetS (10.9 %) groups did vigorous-intensity recreational activities.

4.4.2 Metabolic-associated blood parameters

The MetS consists of several individual risk factors, including abdominal obesity, hypertension, IR/hyperinsulinaemia and impaired glucose homeostasis, and dyslipidaemia (Alberti *et al.*, 2006; Alberti *et al.*, 2009; Jeon *et al.*, 2011; Crowther & Norris, 2012). Although the exact pathophysiology of each of these components and the sum of the individual risk factors remain elusive, studies agree on the individual components of the MetS (Erasmus *et al.*, 2012; Klug *et al.*, 2012; Camargo *et al.*, 2014). Each of these components (WC, SBP, DBP, and glucose and TG levels) were significantly higher, while HDL-c levels were significantly lower in the MetS compared to the non-MetS women in the current study (Table 3.1). Our findings therefore confirm the data obtained from other studies (Nigam *et al.*, 2009; Bremer *et al.*; 2011; Esteghamati *et al.*, 2011; Atanassova *et al.*, 2014). This cluster of metabolic abnormalities increases the risk of CVD, T2DM, lifestyle-associated cancers, and mortality through its effects on hormonal, inflammatory, and growth factor pathways (Kuchiba *et al.*, 2012; Reeves *et al.*, 2012; Peer *et al.*, 2015).

4.4.2.1 Waist circumference

Waist circumference is known to contribute to the increased prevalence of the MetS, including obesity, and in agreement, the current study noted that WC was significantly higher in all groups compared to the NMetS- group (Figure 3.6A). This finding was similar to that observed by Gómez-Ambrosi *et al.* (2012) and Motie *et al.* (2014). Waist circumference was significantly greater in the obese compared to the overweight groups with and without the MetS (Figure 3.6A), which is not an unexpected finding, since obesity is characterised by excessive lipid storage, adipocyte hypertrophy, and weight gain (Donohoe *et al.*, 2010; Blüher & Mantzoros, 2015; Mendonça *et al.*, 2015).

The current study also noted a statistical trend between the WC measurements of the OWMetS- and OWMetS+ groups. The WC is used as a proxy measure of VAT, and in agreement, we reported significantly strong positive associations between WC and VAT %, and VAT:SAT in both obese groups (Appendix J, Table J.1). Furthermore, a significantly strong negative relationship was observed between WC and SAT % in these groups. Increasing VAT leads to an increased risk of the MetS, since increased abdominal fat is associated with increased lipolysis, and therefore increased FFAs in the bloodstream (Evans *et al.*, 2011; Aballay *et al.*, 2013). This reduces insulin sensitivity and impairs insulin signalling, while also increasing insulin synthesis and -secretion (Aballay *et al.*, 2013). Although insulin levels were significantly higher in the MetS

compared to the non-MetS group of the current study, no other significant differences were observed for insulin levels of the obese subgroups (Table 3.1 & Appendix K).

4.4.2.2 Systolic- and diastolic blood pressure

The MetS is associated with arterial hypertension, possibly due to the association between abdominal obesity and IR (Jennings *et al.*, 2009; Jeon *et al.*, 2011; Peer *et al.*, 2013, Helelo *et al.*, 2014; Kaur, 2014). An earlier study by Al-Daghri *et al.* (2013) observed positive relationships between BP, BMI, and WC. Studies by Marques-Vidal *et al.* (2010) and Gómez-Ambrosi *et al.* (2012) noted that SBP and DBP were significantly higher in obese than in normal weight individuals, while Motie *et al.* (2014) found no significant differences for SBP or DBP with increasing BMI. Similar to the findings of Motie *et al.* (2014), the current study did not observe any significant difference for SBP or DBP with increasing BMIs (Figure 3.6B & 3.6C). Increasing body weight is often associated with increased BP, for which several mechanisms have been proposed to explain this association (Wildman *et al.*, 2008; Jeon *et al.*, 2011; Peer *et al.*, 2013; Abu-Farha *et al.*, 2014). One such mechanism suggests that obesity causes microvascular dysfunction and abnormal vascular tone, which leads to improved vasoconstriction and reduced vasodilation, and ultimately increased BP (de Boer *et al.*, 2011; Peer *et al.*, 2013; Helelo *et al.*, 2014; Kaur, 2014).

Hyperinsulinaemia might also be responsible for the high prevalence of hypertension observed in our population, since the study by Al-Daghri *et al.* (2013) noted a significant positive association between BP and HOMA-IR. This is possible, since hyperinsulinaemia increases sodium resorption, leads to vasodilation and volume expansion, and ultimately increases BP (Kirk & Klein, 2009). Insulin levels, and SBP and DBP were all higher than the IDF cut-off values for all the respective groups in the current study (except SBP for the OBMetS-) and this may explain why no significant differences were observed for either SBP, DBP, insulin, and glucose levels with increasing BMI (Appendix K).

One also has to consider the sympathetic nervous activity that is associated with obesity, and which often leads to hyperinsulinaemia and counteracts vasodilation (Aballay *et al.*, 2013; Canale *et al.*, 2013). Alterations in the sympathetic nervous system is part of the pathophysiology of the MetS, and considering its effect on cardiac output, peripheral vascular resistance and renal sodium handling, these factors have been thought to contribute to hypertension *via* its downstream effects (Canale *et al.*, 2013). The current study found SBP to be significantly higher in the OBMetS+ compared to the OBMetS- group (Figure 3.6B), which is in line with the mechanism proposed by Canale *et al.* (2013). Considering that adipocytes produce small quantities of angiotensin, thereby affecting the RAAS that contribute to

hypertension (Aballay *et al.*, 2013), the fact that WC and BMI were significantly higher in all the respective groups compared to the NMetS- group (Figure 3.6A & 3.7A) might explain the overall high SBP and DBP in the current study population.

4.4.2.3 Blood glucose and insulin levels

Studies by Marques-Vidal *et al.* (2010) and Gómez-Ambrosi *et al.* (2012) found exponentially higher glucose and fasting insulin levels with increasing BMI. However, Motie *et al.* (2014) and the current study did not observe significant differences for glucose with increasing BMI (Figure 3.6D), while insulin levels were only significant higher in the OWMetS+ compared with the OWMetS- group (Appendix K). The individual components of the MetS may have played a role, since a positive relationship exists between IR and abdominal obesity (Jennings *et al.*, 2009; de Boer *et al.*, 2011). The proposed mechanism highlights the association between obesity and adipose tissue dysfunction, including its ability to increase the secretion of pro-inflammatory adipokines, which contribute to IR (Shah *et al.*, 2012; Shah *et al.*, 2014; Yeoh *et al.*, 2015). The current study observed several significant differences for WC between the respective groups (Figure 3.6A), which makes it possible that abdominal obesity may have affected glucose and insulin levels.

Literature suggests that IGF-1 plays a role in glucose homeostasis, since increased insulin levels decrease IGFBP levels that could increase IGF-1 bioavailability (Donohoe *et al.*, 2012; Doyle *et al.*, 2012; Rajpathak *et al.*, 2012; Sierra-Johnson *et al.*, 2015). The IGF-1 concentration in the current study was only significantly higher in the OBMetS+ women compared with the NMetS- women, which suggests that IGF-1 may have played a role (Figure 3.15A). Adipokines (such as leptin) function to regulate energy balance and glucose homeostasis; however, obesity and the MetS are associated with dysregulation of adipokines (Yun *et al.*, 2011; Motie *et al.*, 2014). In agreement, Al-Daghri *et al.* (2013) also reported a significant positive association between the HOMA-IR and leptin levels. Dysregulation of leptin synthesis affects insulin signalling and glucose homeostasis, which may lead to hyperglycaemia (Deng & Scherer, 2010; García-Jiménez *et al.*, 2015). Albeit so, the current study did however not observe any significant differences for either glucose or leptin levels in the respective groups (Figure 3.15B).

4.4.2.4 Lipid profile

Another distinctive component of the MetS is dyslipidaemia, which may contribute to atherosclerotic lesions and CVD (Shapira, 2009). Studies by Jeon *et al.* (2011) and Gómez-Ambrosi *et al.* (2012) noted a more atherogenic lipid profile (significantly higher total cholesterol, LDL-c and TGs levels, and lower HDL-c levels) in obese compared to normal weight persons.

This especially places the obese group at an increased risk of dyslipidaemia, the MetS, and CVD (Jeon *et al.*, 2011; Gómez-Ambrosi *et al.*, 2012).

Insulin resistance may also contribute to atherogenic dyslipidaemia, since IR results in increased lipolysis, which in turn elevates TG levels by increasing VLDL production and -secretion, as well as by increasing HDL-c catabolism (Sumner *et al.*, 2005; Ginsberg & Karmally, 2009; Kirk & Klein, 2009; Jeon *et al.*, 2011). Studies also found that low HDL-c levels were significantly associated with an increased risk of IR in women (Jennings *et al.*, 2009; Delisle *et al.*, 2013). This might explain why HDL-c levels were significantly higher in the OWMetS- compared to the OWMetS+ group, since the only significant difference for insulin levels was also observed between these two groups (Figure 3.6E & Appendix K). Triglyceride levels were also significantly higher in the OWMetS+ compared with the OWMetS- women (Figure 3.6F).

The results of the present study did not indicate significant differences in the lipid profiles of the normal and obese groups; however, both HDL-c and TG levels were significantly lower and higher in the OWMetS+ compared to the NMetS- group, respectively (Figure 3.6E & 3.6F). These findings were similar to that reported by Gómez-Ambrosi *et al.* (2012) in persons without the MetS. However, the study by Gómez-Ambrosi *et al.* (2012) also found a significant increase in total cholesterol and LDL-c levels with increasing BMI. In contrast, the study by Motie *et al.* (2014) did not corroborate these findings, findings that were also not evident in our study. Delisle *et al.* (2013) noted that overweight and obesity, as measured by WC, is associated with low HDL-c levels; therefore, obesity may have played a role in the lipid profiles of the women of the current study, since increasing BMI was associated with increasing WC (Figure 3.6A).

4.4.2.5 Confounding factors for the metabolic syndrome components

Gender, ethnicity, age, medical history, lifestyle choices, stress, and level of urbanisation (Umscheid *et al.*, 2010; Jeon *et al.*, 2011; Delisle *et al.*, 2013; Peer *et al.*, 2013) were all confounding factors in the current study and may explain why our findings for some of the individual components of the MetS differ from that observed in previous studies.

Gender

Several studies have reported that the MetS prevalence is much higher in women compared to men (Garrido *et al.*, 2009; Motala *et al.*, 2011; Gyakobo *et al.*, 2012; Shah *et al.*, 2014). A possible explanation is that women have higher body fat, total adiposity, and insulin sensitivity compared to men, and this may be due to hormonal and adipose tissue distribution differences

(Rochlani *et al.*, 2015). The current study only included female participants, which may have influenced the findings.

Ethnicity

The prevalence of the components of the MetS has also been found to differ between ethnic groups. For example, hyperglycaemia was more common among Black than Caucasian women (Evans *et al.*, 2011). Ethnic background, reported by Jennings *et al.* (2009) and Evans *et al.* (2011), was associated with elevated TG levels among Caucasian women compared to Black women. Jennings *et al.* (2009) also suggested that genetic differences between different ethnic groups were partially related to the different lipid profiles. Caucasian, Coloured, and Black women were included in the current study, and cultural influences may have played a role in the prevalence of overweight and obesity in this population. Overweight and obesity are related to all the individual components of the MetS, and ethnicity may have affected these parameters. Moreover, cultural influences are associated with a larger body size in Black women, since it reflects happiness and health (Holdsworth *et al.*, 2004; Kaduka *et al.*, 2012; Peer *et al.*, 2015), and this may contribute to the significantly higher prevalence of obesity among women.

Age

Prevalence of the MetS components also increase with ageing, which was demonstrated in a study by Peer *et al.* (2013), where a higher prevalence for hypertension was noted in the older group (25-34 years *vs* 65-74 years). Ageing is associated with hypertension due to age-associated arterial thickening and increased arterial resistance (Helelo *et al.*, 2014). Another factor to consider is oestrogen and its beneficial effects on BP; however, the age-related decline in oestrogen contributes to hypertension (Hilliard *et al.*, 2013; Rochlani *et al.*, 2015). The ageing-associated decline in oestrogen is also associated with adipose tissue redistribution (Alkerwi *et al.*, 2011; Camhi *et al.*, 2011), and in agreement Senekal *et al.* (2015) also reported that abdominal obesity was associated with ageing. The effects of ageing must also be considered in the current study population, since the MetS group (mean age 40.2 years) was significantly older than their non-MetS counterparts (mean age 35.0 years) (Table 3.1).

Diet

The unhealthy Westernised diet, which mainly includes red and processed meat, saturated and *trans*-fat, and low intake of dietary fibre, fruit and vegetables, is associated with the MetS, and its individual components (de Oliveira *et al.*, 2012; Shab-Bidar *et al.*, 2014; Grosso *et al.*, 2015; Senekal *et al.*, 2015). Poor lifestyle choices, including increased salt intake, contribute to the increased prevalence of hypertension, since sodium homeostasis is disturbed (Sheng, 2009; Helelo *et al.*, 2014). Dietary fat intake such as SFA and *trans*-fats causes lower HDL-c and

higher TG levels *via* its effect on insulin levels, which lead to increased lipolysis (Hosseinpour-Niazi *et al.*, 2016). The women of the current study consumed several food products that contain SFAs and *trans*-fats and this may have influenced the different components of the MetS (Figure 3.16).

Smoking

Smoking may have unfavourable effects on the components of the MetS. For example, smoking affects BP, since it is associated with endothelial injury, increased sympathetic activity induced by nicotine, increased inflammation, increased arterial stiffness, and changes in lipid profile (Malekzadeh *et al.*, 2013). Although the majority of women in the current study in both the MetS and non-MetS groups were non-smokers (Appendix I.1), factors such as under-reporting and the lack of information on smoking duration, number of cigarettes smoked, and the effects of previous smoking may be confounding factors.

Physical activity

Physical activity is another lifestyle factor that is associated with the MetS. Studies suggest that physical activity is protective against hypertension (Malekzadeh *et al.*, 2013; Peer *et al.*, 2013), possibly due to its ability to induce weight loss, increase metabolic rate and fat metabolism, and reduce IR, plasma renin, and vascular tone (Golbidi *et al.*, 2012; Malekzadeh *et al.*, 2013). A smaller proportion of MetS (50.0 % for moderate-intensity and 17.7 % for vigorous-intensity) women in the current study participated in moderate- and vigorous-intensity activities at work compared to their non-MetS counterparts (55.6 % for moderate-intensity and 20.0 % for vigorous-intensity) (Appendix I.2). More women in the MetS group (76.5 % *vs* 69.6 %) participated in moderate-intensity recreational activities, which may indicate over-reporting of physical activity in the women of the MetS group.

4.4.3 Anthropometric parameters

For the present study, all anthropometric indices of adiposity (BMI) and abdominal adiposity (WC, W:H, W:Ht, and SAD) were significantly higher in the MetS group (Table 3.1). Studies by both Bremer *et al.* (2011) and Atanssova *et al.* (2014) showed BMI and WC to be significantly higher, while Cheong *et al.* (2015) and Peer *et al.* (2015) found not only BMI and WC, but also W:H to be significantly higher in MetS participants. Despite the lack of statistical significance between the HC measurements of the MetS and non-MetS groups, significant differences were noted between the NMetS- group and all the subgroups, as well as the overweight and obese groups (Figure 3.7B). These findings are in agreement with that of Gómez-Ambrosi *et al.* (2012), who found that WC and HC measurements, and the W:H were significantly greater in obese- compared with normal weight persons. The W:H was only significantly higher in the OBMetS+

compared to the NMetS- group, while a statistical trend was noted between the OWMetS+ and NMetS- groups (Figure 3.7C).

The WC, HC and W:H are surrogate measures of adipose tissue distribution, particularly the more pathogenic visceral compartment (Willet & Hu, 2013; Anunciaçao *et al.*, 2014; Cheong *et al.*, 2015). Yim *et al.* (2010) also found that the SAD is indicative of visceral obesity, regardless of age, gender, degree of obesity or other metabolic parameters. This is in agreement with the current study where both obese groups presented with a significantly greater SAD compared to their overweight counterparts (Figure 3.7D). The SAD of the OWMetS+, OBMetS+ and OBMetS- was also significantly greater compared to the NMetS- group. Furthermore, a strong positive relationship was established between the SAD and VAT % in both obese groups (Appendix J, Table J.2), which confirms the findings reported by Yim *et al.* (2010).

Although the IDF definition do not include the SAD and W:Ht, these anthropometric parameters have been shown to correlate with the components of the MetS, including TGs, glucose levels, and BP (Schneider *et al.*, 2011; Ware *et al.*, 2014). Visceral adiposity is also associated with several adverse metabolic alterations and lifestyle-associated cancers through its effects on inflammatory cytokine production that promotes carcinogenesis (Kang *et al.*, 2010; Yamamoto *et al.*, 2010; Yim *et al.*, 2010; Donohoe *et al.*, 2011; Doyle *et al.*, 2012; Anunciaçao *et al.*, 2014; Carlsson *et al.*, 2014; Vasques *et al.*, 2015). Adipose tissue metabolism is closely related to the complications of obesity preceding the MetS, and NCDs such as T2DM, CVD, and certain lifestyle-associated cancers (Evans *et al.*, 2011; Jeon *et al.*, 2011, Gómez-Ambrosi *et al.*, 2012; Peer *et al.*, 2013, Abu-Farha *et al.*, 2014; Helelo *et al.*, 2014; Almquist *et al.*, 2015; Miglani *et al.*, 2015).

Under normal physiological conditions, adipose tissue will neutralise and store nutritional overload to protect the body from IR (Esteves & Dias-Peixoto, 2015). In contrast, the obese state is associated with adipose tissue dysfunction and its associated effect in altering adipokine- and cytokine secretion (Kranendonk *et al.*, 2015). This causes a state of low-grade chronic inflammation, and macrophage mobilisation and infiltration, which lead to hyperinsulinaemia, IR, dyslipidaemia, and hypertension that ultimately contribute to the development of the MetS (Bremer *et al.*, 2011; Esteves & Dias-Peixoto, 2015; Kranendonk *et al.*, 2015). Thus, since the MetS group presented with increased BP, and TG and insulin levels (Table 3.1, Figure 3.6 & Appendix K), we cannot rule out the involvement of the inflammatory and immune systems that accompanies the obese state, which may also have contributed here.

Age might be considered a possible confounding factor in the associations between the MetS and WC, HC, and W:H, since Al-Daghri *et al.* (2013) observed positive relationships between age and the W:H in non-MetS normal weight persons between the ages of 22 and 67 years. Cheong *et al.* (2015) proposed that this was due to the age-associated changes in total- and regional adipose tissue distribution. In agreement, the current study observed a positive statistical trend between age and W:H for the NMetS- group (Appendix K, Table J.3). Although the MetS group (mean age 40.2 years) of the current study was also significantly older than the non-MetS group (mean age 35.0 years) (Table 3.1), no other significant positive relationships were observed between age and W:H.

A limitation of using WC, HC, and SAD is that measurements may be inaccurate and overestimated due to the standing position of the participant and/or the looseness of the abdominal- and gluteal muscles (Willet & Hu, 2013; Anunciaçao *et al.*, 2014). Different studies have also used different anatomical locations to measure the WC, HC, and SAD, and different cut-off values may have been used, which complicates the comparison of studies (Anunciaçao *et al.*, 2014; Hoebel *et al.*, 2014). In obese participants, it may be especially difficult to locate the anatomical landmarks to take the measurements due to the accumulation of adipose tissue around this site (Steward & Sutton, 2012).

4.4.4 Bioelectrical impedance analysis characteristics

The MetS group showed significantly greater VAT area, VAT %, and VAT:SAT, while SAT % was significantly lower (Table 3.2). These findings are similar to studies by Fox *et al.* (2007), Kim *et al.* (2011), and Yeoh *et al.* (2015) who also showed that in women, an increasing VAT:SAT was significantly associated not only with the MetS, but also with all of the individual components of the MetS. In agreement, the current study noted significant positive associations between VAT:SAT and WC for both obese groups, while a positive correlation was also noted between VAT:SAT and SBP for the OBMetS+ group (Appendix J, Table J.1 & Table J.4). Statistical trends were also observed for VAT:SAT and HDL-c, and VAT:SAT and LDL-c for OWMetS- women. The VAT and SAT differ in anatomical location, LPL activity, and cytokine secretion profiles, and therefore its association with cardiometabolic diseases (Liu *et al.*, 2010b), and this may explain why the MetS group also presented with significantly greater VAT area, VAT % and VAT:SAT.

Although studies on the role of SAT in the pathogenesis of the MetS are mostly inconsistent, it was suggested that increased SAT might lead to hepatic- and skeletal muscle fat accumulation with associated IR that contributes to the pathogenesis of the MetS (Pou *et al*., 2009; Liu *et al.*, 2010b; Bremer *et al.*, 2011). Others found that the SAT depot is the least active adipose tissue

depot with lower macrophage infiltration, and adipokine secretion (Pou *et al*., 2009; Kranendonk *et al.*, 2015), since the visceral compartment is more metabolically active, thereby secreting larger quantities of pro-inflammatory adipokines, which is directly associated with IR (Shah *et al.*, 2014; Mendonça *et al*., 2015; Yeoh *et al*., 2015). Moreover, VAT is regarded as more lipolytically active, promoting the release of FFAs into the portal circulation and increasing hepatosteatosis (Esteves & Dias-Peixoto, 2015; Kranendonk *et al.*, 2015). This directly affects hepatic glucose- and lipid metabolism, because increased visceral adiposity is associated with impaired β-cell function, and subsequent reduced insulin sensitivity and IR (Jennings *et al*., 2009; Gletsu-Miller *et al*., 2013). This is also evident in the current study where significantly higher insulin levels were observed in the MetS compared with the non-MetS group, and the OWMetS+ group presented with significantly higher insulin levels compared to the OWMetS- group (Table 3.1 & Appendix K).

In a state of positive energy balance, excess FFAs are stored in the SAT; however, during a state of caloric excess, remodelling of the adipose tissue takes place *via* adipocyte hypertrophy and -hyperplasia (Yeoh *et al.*, 2015). In the obese state, adipose tissue remodelling becomes pathologic, since the lack of adequate adipocytes and the limited capacity to store adipose tissue subcutaneously, results in spill over into the ectopic (visceral) fat compartment (Pou *et al.*, 2009; Yeoh *et al.*, 2015). This state is characterised by hypoxia, angiogenesis, inflammation, and concomitant IR (Bremer *et al.*, 2011; Vykoukal & Davies, 2011), a mechanism supported by the findings of several studies that indicated that increasing BMI is associated with increasing VAT- and SAT areas (Camhi *et al*., 2011; Motala *et al*., 2011; Motie *et al*., 2014; Shah *et al*., 2014; Vasques *et al*., 2015). Our results also showed that the SAT areas were significantly greater in the OWMetS+, OBMetS+ and OBMetS- groups compared with the NMetS- group, while the VAT area was also significantly greater in the OWMetS- compared with the NMetS- group (Figure 3.8). Additionally, VAT and SAT areas were also greater in the OBMetS- compared to the OWMetS- groups, while a statistical trend was observed for the OBMetS+ and OWMetS+ groups. The SAT % was significantly higher in the NMetS- compared with all the respective subgroups, while both overweight groups presented with significantly higher SAT % than their obese counterparts (Figure 3.9A). The opposite was true for VAT % and VAT:SAT (Figure 3.9B & 3.9C).

The area of adipose tissue distribution is related to metabolic dysfunction, because it was shown that visceral adiposity is more pathogenic than subcutaneous adiposity (Liu *et al.*, 2010b; Shah *et al.*, 2014; Kranendonk *et al.*, 2015). The distribution of adipose tissue is also a key factor in the pathophysiology of the MetS (Jeon *et al.*, 2011; Camargo *et al.*, 2014; Alberti *et al.*, 2006; Crowther & Norris, 2012). Adipose tissue is either stored in the subcutaneous- or visceral

compartments to participate in metabolic pathways such as lipid- and glucose metabolism (Kranendonk *et al.*, 2015). If one assumes that anthropometric measures are correlated with more direct measures of obesity, such as SAT and VAT (Camhi *et al.*, 2011), BMI might be considered a useful surrogate to describe obesity status. This is possible, since VAT and SAT have been related to metabolic dysfunction, and both are associated with BMI and WC (Pou *et al.*, 2009; Yim *et al.*, 2010; Camhi *et al.*, 2011; Kaess *et al*., 2012; Yeoh *et al.*, 2015). In agreement, the present study also found positive associations between BMI and VAT:SAT, and BMI and VAT % in the obese subgroups (Figure 3.17C & 3.17F). Additionally, positive relationships were also revealed for WC and VAT:SAT, WC and VAT %, SAD and VAT:SAT, and SAD and VAT % in the obese subgroups (Appendix J, Table J.1 & J.2). Opposing results were obtained for SAT % (Figure 3.17I and Appendix J, Table J.1 & J.2). This is in agreement with the findings of Yeoh *et al.* (2015), who reported that during caloric excess, the cellular characteristics of SAT changes reduce its ability to expand, and excess calories are stored in the visceral compartment. These findings also confirm that increasing BMI is associated with increasing VAT, which is associated with an increasing risk of CVD, and several lifestyle-associated cancers (Kuchiba *et al.*, 2012; Wiedman *et al.*, 2013; Lindkvist *et al.*, 2014; Kawai *et al.*,2013; Yeoh *et al.*, 2015).

The metabolic changes associated with obesity, such as IR, hypertension, and dyslipidaemia can also contribute to increased cancer risk (Jeon *et al.*, 2011; Peer *et al.*, 2013; Helelo *et al.*, 2014). Abdominal obesity can specifically increase the risk of cancer, since adipose tissue can act in an autocrine or paracrine manner to alter the insulin/IGF-1 pathway, and increase inflammatory markers and the production of adipokines such as leptin that promote carcinogenesis (Donohoe *et al.*, 2011; Donohoe *et al.*, 2012; Doyle *et al.*, 2012). The current study also noted that several measures of abdominal obesity, and IGF-1 and leptin levels were significantly higher in the MetS group (Table 3.1 & 3.4).

The degree of obesity is not the only factor responsible for the association between obesity and IR, but the site of adipose tissue accumulation may also play a role (Vasques *et al.*, 2015). Liu *et al.* (2010b) found that the VAT compartment is a much stronger correlate of the components of the MetS compared to the SAT compartment. In agreement, Evans *et al.* (2011) found that VAT is associated with increased sympathetic activity and increased angiotensin expression, which contributes to increased BP. Visceral adipose tissue accumulation may also have contributed to hypertension in the current population, since MetS women presented with significantly higher VAT area and -%, VAT:SAT, SBP and DBP (Table 3.1 & 3.4). Kaess *et al*. (2012) also reported significant positive associations between the VAT:SAT and several cardiometabolic risk factors, including the MetS components.

The current study found statistical trends between TG levels and VAT %, and VAT:SAT in the NMetS- group (Appendix J, Table J.5). Additionally, a strong negative trend was observed between SAT % and TG levels. This is in agreement with earlier findings by Fox *et al.* (2007), where obesity and the obesity-associated chronic inflammation alters lipoprotein metabolism, and IR in the liver, thereby reducing VLDL and TG catabolism and increasing HDL-c catabolism.

Several confounding factors may influence adipose tissue distribution. For example, Shah *et al*. (2012) reported that women have significantly higher SAT and lower VAT compared to men. The current study only included women, and these findings may therefore not be applicable to men. Although the principles underlying the association between VAT and SAT, and gender is unclear, this association may be related to the regulatory effects of oestrogen on metabolism, and lipogenesis (Kaess *et al*., 2012). Ethnicity is another factor that may influence adipose tissue distribution, since Black women presented with lower VAT compared to their Caucasian counterparts for the same WC measurement (Jennings *et al.*, 2009; Camhi *et al.*, 2011). Age may also influence adipose tissue distribution, since ageing is associated with the loss of SAT and gain of VAT. Yim *et al.* (2010) demonstrated this mechanism by reporting significantly higher VAT and lower SAT in older women (75-84 years) compared to younger counterparts (35-44 years). Although all of these factors mentioned above may have influenced the BIA findings of the current study, we did not specifically assess the association between these factors and the different measures of adipose tissue distribution.

4.4.5 Red blood cell membrane fatty acid profile

Comparison of the FA profiles of healthy individuals to those diagnosed with the MetS indicated a possible link between the MetS and specific FAs (Kabagambe *et al.*, 2008; Novgorodtseva *et al.*, 2011; Zong *et al.*, 2013). Body mass index has a modifying effect on the relationship between the individual components of the MetS and FA composition. This was demonstrated by Klein-Platat *et al.* (2005), who reported that FA levels are directly associated with weight status, since SFAs were significantly higher, and PUFA levels were significantly lower in overweight individuals respectively. Fatty acids are also regarded as the major link between obesity, IR, and cancer, since increasing body weight is associated with hyperinsulinaemia/IR, and increased IGF-1 levels that creates a pro-tumorigenic environment (Boden, 2009; Doyle *et al.*, 2012).

4.4.5.1 Saturated fatty acids

Evidence suggest that the MetS is positively correlated with SFAs, since MetS groups presented with significantly higher SA levels compared to healthy controls (Nigam *et al.*, 2009; Novgorodtseva *et al.*, 2011; Žák *et al.*, 2014). The results of the current study did not report any significant differences in PA or SA levels in the MetS and non-MetS groups, or any of the

respective subgroups (Table 3.3, Figure 3.13A & 3.14A). Factors such as IR, obesity, and dyslipidaemia are positively correlated with FA levels, which may explain the discrepant findings – various studies reported even higher HOMA-IR, insulin, TG and LDL-c levels, and WC measurements (Nigam *et al.,* 2009; Novgorodtseva *et al.,* 2011; Žák *et al.,* 2014) compared to the present study. Women in the MetS group of the current study did however present with significantly higher glucose and insulin levels compared to the non-MetS group (Table 3.1, Figure 3.6 & Appendix K).

Saturated fatty acids and insulin resistance

Insulin resistance may have played a role in the non-significant findings for the SFAs, SA and PA, in the current study (Table 3.1, Figure 3.13A & 3.14A). This is possible since the MetS is characterised by elevated glucose and insulin levels, and this state is associated with enhanced hepatic *de novo* lipogenesis and reduced FA oxidation (Sethom *et al.*, 2011). Although various studies reported positive relationships between glucose levels and SFAs (Kabagambe *et al.,* 2008; Patel *et al.,* 2010), the current study did not observe any significant difference in SFA or glucose levels between the subgroups (Figure 3.6, 3.13 & 3.14). Dietary fat quality may influence glucose metabolism and the development of IR, since the consumption of SFAs is associated with reduced insulin sensitivity (Nigam *et al.*, 2009; Astrup *et al.*, 2011; Patel *et al.*, 2010). One of the proposed mechanisms for this association is that SFAs have adverse effects on cell membrane function due to alterations in membrane fluidity, ion permeability, and insulin receptor binding and -affinity (Patel *et al.*, 2010). The current study has shown this to be true, since here, relatively large quantities of SFA fat were consumed, and insulin levels were also elevated (Table 3.5 & Appendix K).

Saturated fatty acids and obesity

A study by Kawashima *et al.* (2009) did not find any significant differences between the SFA levels of normal and obese persons, similar to the results for SFAs observed in the present study (Figure 3.13A & 3.14A). However, a recent study by Tremblay-Franco *et al.* (2015) found that the MetS obese group presented with higher SA levels and decreased Δ-9 SCD 2 activity compared to the non-MetS normal weight participants. Pickens *et al.* (2015) corroborated these findings; however, the current study did not observe any statistically significant differences for Δ-9 SCD 2 enzyme activity or n-9 SI between the respective groups (Figure 3.14C & 3.14D). Different factors could account for the discrepant results, such as the specific MetS definition used, and the fact that Tremblay-Franco *et al.* (2015) matched participants for age and gender. Furthermore, geographical region may have played a role, and the large variability in sample

sizes used in Tremblay-Franco *et al.'s* (2015) study compared to the current one (n=75 *vs* n=13 for the MetS obese group, and n=210 *vs* n=16 for the non-MetS normal weight) (Figure 3.5).

A dairy intervention study by Abdullah *et al.* (2015) found that although SFAs are predominant in dairy products, SA levels were not significantly different between the control and intervention groups. In agreement, women of the current study consumed relatively large quantities of SFA-containing foods, including red meat, lamb, pork, chicken, and whole milk products, although SFA levels did not differ significantly between the groups (Figure 3.16).

Saturated fatty acids and dyslipidaemia

Some studies have also reported on the association between SFAs and dyslipidaemia; however, results have been conflicting, possibly due to the diverse effects of specific SFA on the lipid profile (Mayneris-Perxachs *et al.*, 2014; Hlavaty *et al.*, 2015; Ruiz-Núñez *et al.*, 2016). The mechanism proposes that changes in lipoprotein synthesis leads to increased LDL-c and TG levels, glucose metabolism enzyme activity, and inflammation (Patel *et al.*, 2010; Astrup *et al.*, 2011; Abdullah *et al.*, 2015). Kim *et al.* (2015) supported this mechanism by reporting a positive association between PA and TG levels. Although TG levels were significantly higher in the OWMetS+ group compared to the OWMetS- and NMetS- groups, no significant differences were noted for PA levels in any of the subgroups in the current study (Figure 3.6 & 3.16).

4.4.5.2 Monounsaturated fatty acids

We did not report any significant differences for the MUFAs (PLA and OA) between the MetS and non-MetS groups, or any of the subgroups in this study (Table 3.3, Figure 3.13B & 3.14B). Although Novgorodtseva *et al.* (2011) reported significantly lower PLA in the MetS group compared to healthy controls, studies by Nigam *et al.* (2009), Sethom *et al.* (2011), and Žák *et al.* (2014) found the opposite to be true. Nigram *et al.* (2009) and Žák *et al.* (2014) also noted that OA levels did not differ between groups with and without the MetS; whereas, Sethom *et al.* (2011) found OA levels to be significantly higher in the MetS group, possibly due to the compensatory upregulation of the Δ-9 SCD enzymes in response to persistently higher SFA levels here. We reported no significant difference in Δ-9 SCD 1 or -2 activities, the n-9 SI, or any of the individual FAs used to calculate these ratios (Table 3.3, Figure 3.13 & 3.14). Since the activities of the Δ-9 SCD desaturase enzymes are under hormonal- and nutritional control (Mikirova *et al.*, 2004), elevated MUFA levels may therefore suggest an increased insulin-induced upregulation of Δ-9 SCD activity to convert SFAs to MUFAs (Sethom *et al.*, 2011). Although insulin levels in the MetS group were significantly higher than the non-MetS group

(Table 3.1), a finding also evident in the overweight subgroups, no significant differences in MUFA levels were observed (Figure 3.13 & 3.14).

Monounsaturated fatty acids and insulin resistance

Increased FFAs are associated with reduced insulin sensitivity and inhibition of glucose uptake that ultimately lead to IR (Boden, 2009; Mendonça *et al.*, 2015). Levels of MUFAs are also positively associated with insulin levels and IR, because insulin stimulates hepatic *de novo* lipogenesis and reduces FA oxidation (Nigam *et al.*, 2009; Sethom *et al.*, 2011; Kurotani *et al.*, 2012; Sertoglu *et al.*, 2014). The only significant difference noted for insulin levels in the current study, were significantly higher insulin levels in the OWMetS+ group compared to the OWMetS- group, and yet MUFA levels did not indicate any significance (Figure 3.13 & Appendix K). Palmitoleic acid is normally not present in the diet and dietary intake may therefore not have played a role; therefore, PLA levels reflect the conversion of PA to PLA *via* the Δ-9 SCD 1 enzyme (Kurotani *et al.*, 2012).

Even though the women in the current study consumed several OA-containing food products, including olive- and sunflower oil, and potatoes (Ginsberg & Karmally, 2009), OA levels still did not differ between any of the groups or subgroups (Table 3.3, Figure 3.14B & 3.16). A possible explanation may involve the use of the three-day food diary, which only collected information on dietary habits three days following the initial visit to the research group, and which is not really comparable to FA levels in the RBC membranes, since it shows medium-term intake instead (± 120 days). The Westernised diet also obtains OA from animal products, which contains large quantities of SFAs, and this makes it likely that SFA intake confounded the OA findings. Women in the current study consumed relatively large quantities of foods containing SFAs, including dairy products (full cream milk and cheese), sunflower oil, and meat products (Figure 3.16).

Monounsaturated fatty acids and obesity

Obesity may also affect MUFA levels (Sethom *et al.*, 2011; Žák *et al.,* 2014), which was evident in a study by Tremblay-Franco *et al.* (2015), where PLA levels were significantly higher in obese women with the MetS. More recently, BMI was positively associated with PLA levels in a study by Dugas *et al.* (2016). This is possible since obesity is associated with reduced FA oxidation and increased lipogenesis (Sethom *et al.*, 2011; Tremblay-Franco *et al.*, 2015). However, the current study did not show any significant differences in PLA levels or any significant associations between PLA levels and W:H in any of the different groups (Figure 3.13B & Appendix J, Table J.6).

Distinct differences in the activity of the Δ-9 SCD 1 enzyme has been reported between normal weight and obese groups, with Δ-9 SCD 1 enzyme activity being significantly elevated in the obese group (Kawashima *et al.*, 2009). In contrast, the present study did not find any significant differences in the Δ-9 SCD 1 enzyme with increasing BMI (Figure 3.13C). While the current study used BMI to define obesity (normal weight: BMI=18.5-24.9 kg/m^2 and obese: BMI≥30.0 kg/m^2), Kawashima *et al.* (2009) defined the normal and obese groups according to WC (normal: WC<85 cm and obese: WC≥85 cm). The WC is a surrogate measure of abdominal obesity, while BMI reflects level of adiposity (Willet & Hu, 2013), and this may explain why Kawashima *et al.* (2009) reported significant findings. These findings may therefore suggest a much stronger association between Δ-9 SCD 1 activity and abdominal obesity, rather than BMI.

Monounsaturated fatty acids and dyslipidaemia

Obesity is associated with increased lipolysis and subsequent increased FFA release, and increased synthesis of glucose, TGs, and VLDL particles that lead to dyslipidaemia (Kawashima *et al.*, 2009; Kurotani *et al.*, 2012; Tremblay-Franco *et al.*, 2015). In agreement, Kim *et al.* (2015) reported strong positive associations between TG levels and PLA, OA, and Δ-9 SCD, which is possible since Δ-9 SCD overexpression is associated with elevated TG production. However, although metabolic parameters of dyslipidaemia (TG and LDL-c levels) and glucose metabolism (glucose and insulin levels) were significantly higher in the MetS groups of the current study, no significant differences in MUFAs were observed (Table 3.1, Figure 3.13 & 3.14). It should however also be noted that the desaturase indices are calculated as product-to-precursor ratios, and this is only an indirect measure of desaturase activity.

4.4.5.3 Omega-3 polyunsaturated fatty acids

Nigam *et al.* (2009) found that MetS participants presented with significantly lower EPA and DHA levels compared to healthy controls. Similar to that reported by Sethom *et al.* (2011), the current study found no significant difference in the EPA or DHA levels in the MetS and non-MetS participants (Table 3.3). The study population of Nigam *et al.* (2009) included individuals with history of acute myocardial infarction, and this disease and its medication may be the reason for the contrasting results, since the use of fibrates have been shown to decrease DHA levels. Nigram *et al.* (2009) further showed that alterations in the Ω-3 FA levels in individuals with the MetS caused a significantly lower Ω-3 index compared to healthy controls. In contrast, the present study did not find a significant difference in the Ω-3 index between the MetS and non-MetS groups (Table 3.3). Considering the association between the MetS and the different classes of Ω-3 FAs (Nigam *et al.,* 2009), it is believed that the individual components of the MetS will also have different effects on the Ω-3 FAs. Insulin resistance may explain the

contrasting findings, since Nigam *et al*. (2009) used the ATP III definition to diagnose the MetS, while the current study used the IDF definition. These definitions differ regarding the cut-off value for impaired glucose regulation (≥6.1 mmol/L for ATP III and ≥5.6 mmol/L for IDF), and therefore the study by Nigam *et al*. (2009) investigated participants with a higher degree of IR. The IR state is associated with enhanced hepatic *de novo* lipogenesis, and reduced FA oxidation, which may be responsible for the negative relationship between IR and Ω-3 FAs (Sethom *et al*., 2011).

Omega-3 fatty acids and insulin resistance

Positive associations have been reported between the components of the MetS and α-LA levels. For example, Cespedes *et al.* (2015) found lower glucose levels among persons with higher α-LA levels, suggesting that α-LA may have beneficial effects on glucose metabolism. The glucose levels of the current study population did not reach statistical significance for any of the respective groups and this might have played a role in the non-significant findings for α-LA levels (Figure 3.6D).

Alpha-linolenic acid, one of the major Ω-3 FAs found in nuts, seeds and some vegetable oils, can be converted to EPA and DHA *via* the desaturase enzymes (DeFilippis & Sperling, 2006). Factors such as LA levels and competitive inhibition of the desaturase enzymes by EPA and DHA may also influence the conversion of α-LA to EPA, and then DHA (DeFilippis & Sperling, 2006). Astorg *et al.* (2008) found that α-LA levels did not correspond with its dietary intake, and therefore reflects the *de novo* biosynthesis of α-LA, which could suggest that the dietary intake of the current study may also not have contributed to the non-significant findings for α-LA between the MetS and non-MetS groups (Table 3.3).

Omega-3 fatty acids and blood pressure

Omega-3 FAs also have favourable effects on BP due to the modulation of membrane fluidity, activities of membrane enzymes and -receptors, and the production of anti-inflammatory eicosanoids (DeFilippis & Sperling, 2006; Nagao & Yanagita, 2008). However, the MetS women in the current study had significantly higher SBP and DBP compared to non-MetS women, which may possibly be due to the low Ω-3 FA levels (Table 3.1). Several mechanisms have been proposed for this association, including changes to the cell membrane, activation of a pro-inflammatory state, and increased vasoconstriction (Novgordtseva *et al.*, 2011). Omega-3 FAs were shown to have beneficial effects on SBP by improving endothelial function, suppressing aldosterone secretion, alterations in plasma viscosity, and by regulating blood fluid volume

through the RAAS (Kabagambe *et al.*, 2008; Cabo *et al.*, 2012; Vanhala *et al.*, 2012). Additionally, DHA can modulate the RAAS to promote apoptosis of vascular smooth muscle (Cabo *et al.*, 2012).

Literature indicates that Ω-3 FAs (specifically EPA and DHA) are precursors of lipoxins, resolvins and protectins, which function to regulate vascular tone, mediate vasoconstriction, and regulate BP (Cabo *et al.*, 2012; Kim *et al.*, 2013). It is therefore not surprising that participants in the highest DHA quartile had a significantly lower risk of developing hypertension (Zhang *et al.,* 2012a). In agreement, correlation analyses of the present study revealed negative relationships between SBP and DHA, DPA, the Ω-3 index, and the Ω-3:Ω-6 for the NMetS- group, although none of these reached statistical significance (Figure 3.18). The relationship between Ω-3 FAs and BP remain controversial, since some studies reported negative associations, while Cespedes *et al.* (2015) did not observe any association between Ω-3 FAs and blood pressure. In contrast, strong positive statistical trends were revealed between SBP and EPA, DHA, the Ω-3 index, and the Ω-3:Ω-6 for the OWMetS+ group in the present study. These findings may be attributed to impaired FA metabolism associated with the MetS, which may accelerate arterial stiffness and cause hypertension (Kim *et al.*, 2013).

Omega-3 fatty acids and obesity

Obesity may also influence the Ω-3 index, although limited scientific evidence exists on the beneficial effects of Ω-3 FAs on obesity (von Schacky, 2011). Some reported a negative associated between Ω-3 FAs and abdominal obesity (Kabagambe *et al.*, 2008; Nagao & Yanagita, 2008), while Tremblay-Franco *et al.* (2015) and the current study did not observe any significant difference in the Ω-3 FA levels of normal and obese persons, regardless of their MetS status (Figure 3.10 & 3.11).

The suggested mechanism for the beneficial effects of Ω-3 FAs on abdominal obesity is that elevated Ω-3 FA levels improve FA β-oxidation, which reduces fat mass and body weight (Clarke *et al.*, 2001; Klein-Platat *et al*., 2005; Kabagambe *et al.*, 2008). This is in agreement with findings by Kawashima *et al.* (2009), who noted lower Ω-3 FA levels in obese compared to normal weight counterparts, albeit in men (mean age 49.3 years for the normal weight group and 51.0 years for the obese group). The study by Kawashima *et al.* (2009) differed from the current study, since male participants in their study were allocated to the control (without any MetS risk factors), abdominal obese (WC≥85 cm) or MetS groups (IDF definition). Female participants in the current study were allocated to the MetS (IDF definition, WC≥80 cm) or non-MetS groups, although participants in the latter group also presented with some of the MetS risk

factors that may have influenced our findings (Figure 3.2 & 3.3). Women of the current study were further categorised according to BMI and not WC like the study by Kawashima *et al.* (2009). These are surrogate measures of obesity and abdominal obesity, respectively, and this may have influenced our findings.

Confounding factors such as age, diet, and Ω-6 FAs may influence Ω-3 FA levels (von Schaky, 2011). Ageing needs to be considered, since it is associated with increased prevalence of obesity, which is associated with abnormal lipid metabolism and this may affect FA levels (Jeon *et al.*, 2011). Although the MetS group of the current study was significantly older (40.2 years *vs* 35.0 years), no significant differences were observed for the Ω-3 FAs (Table 3.1 & 3.4). Eating frequency, fat intake, and meat- and snack consumption are just some of the dietary factors that may play a role in the development of obesity, and obesity-associated changes to the FA profile (Jeon *et al.*, 2011). A dairy intervention study by Abdullah *et al.* (2015) found that the intervention group presented with significantly higher plasma DPA levels compared to the control group. Dietary intake of the women in the current study revealed three dairy products in the top ten food products consumed, which may have influenced our findings (Figure 3.16). However, dietary intake questionnaires are susceptible to self-reported errors resulting in either over- or underestimation of dietary consumption.

Body mass index is inversely associated with Ω-3 FAs, and moreover, it is also inversely associated with SAT (Pou *et al*., 2009; Yim *et al*., 2010; Camhi *et al*., 2011; Doyle *et al*., 2012; Shah *et al*., 2014; Yeoh *et al*., 2015). In addition, a positive relationship was reported between SAT and Ω-3 FAs (Fekete *et al*., 2015). Heemskerk *et al.* (2015) recently showed that SAT of obese women contained significantly higher EPA, DPA, and DHA levels to reduce obesity-associated inflammation, since Ω-3 FAs have anti-inflammatory properties. Accordingly, the present study also observed significant positive correlations between the SAT area and the Ω-3 index in OWMetS+ and OBMetS- women, and also between SAT and the Ω-3:Ω-6 in the OWMetS+ and OBMetS+ women (Figure 3.19). Regardless of the relatively low Ω-3 FA levels in adipose tissue, adipose-specific beneficial effects include prevention of adipose tissue hyperplasia and hypertrophy, and the improvement of adipose tissue inflammation (Cespedes *et al.*, 2015). This is in direct contrast to the pro-inflammatory effects of the Ω-6 FAs (DeFilippis & Sperling, 2006; Odegaard, 2009).

4.4.5.4 Omega-6 polyunsaturated *fatty acids*

Arachidonic acid, commonly found in animal meat, is one of the most prominent Ω-6 FAs in the human diet (DeFilippis & Sperling, 2006). Despite this, the link between the MetS and AA still

remains unclear, since the current study (Table 3.3), as well as others (Nigam *et al.*, 2009; Žák *et al.*, 2014) report no difference in AA levels. However, the studies by Sethom *et al.* (2011) and Novgorodtseva *et al.* (2011) observed significantly lower AA levels in the MetS compared to the non-MetS group. Nigam *et al*. (2009) (ATP III definition), Žák *et al.* (2014) and the current study (both used the IDF definition) reported similar findings, yet different definitions of the MetS were used. Interestingly, Sethom *et al.* (2011) also used the IDF definition, yet different results were obtained. Novgorodtseva *et al.* (2011) used the AHA/NHLBI definition of the MetS, which only differ from the IDF regarding WC cut-off values (females WC≥88 cm for AHA, and WC≥80 cm for IDF). The contrasting findings between the current study and that of Novgorodtseva *et al.* (2011) may also be a result of the group allocation of participants, since Novgorodtseva *et al.* (2011) characterised participants with MetS and normal insulin levels, or controls. The current study on the other hand, identified participants with the MetS (WC≥80 cm, and any two or more MetS risk factors) or without the MetS (WC<80 cm, regardless of the presence of any MetS risk factors), and participants with elevated insulin levels were present in both groups. The inverse association between insulin and AA levels may explain the contrasting results, since insulin levels were much higher in the current population (34.4 and 15.6 mIU/L, Table 3.1) compared to the population used in the study by Novgorodtseva *et al.* (2011) (18.5 and 8.1 mIU/L). Studies have reported significant inverse relationships between insulin and Ω-6 FAs (Kabagambe *et al.*, 2008; Novgorodtseva *et al.*, 2011). Elevated insulin levels and the degree of IR in the current study population may consequently have contributed to the low Ω-6 levels and the non-significant findings, since the Ω-6 FAs are precursors of pro-inflammatory eicosanoids, and this increases the risk of IR through inflammatory processes (Spector, 2000; Strandvik, 2011; Shah *et al.*, 2012; Hussein, 2013; Suburu *et al.*, 2013).

Omega-6 fatty acids and insulin resistance

Insulin and AA levels are inversely associated due to the reduced desaturation-elongation cascade in IR, which worsens as IR increases (Nigam *et al.*, 2009; Kim *et al.*, 2013). Degree of obesity and distribution of adipose tissue may also play a role in the levels of different Ω-6 FAs (Kabagambe *et al.*, 2008; Kim *et al.*, 2013). Visceral obesity is associated with IR and therefore, AA levels are negatively associated with VAT % (Kim *et al.*, 2013; Vasques *et al.*, 2015). However, the present study did not observe any significant associations between AA levels and VAT % (Appendix J, Table J.7). Insulin levels were also significantly higher in the OWMetS+ compared with the OWMetS- group (Appendix K).

Omega-6 fatty acids and obesity

Comparing AA levels of normal weight persons with and without the MetS indicated significantly lower AA levels in the latter group (Tremblay-Franco *et al.*, 2015). Additionally, the obese non-MetS group presented with significantly higher AA levels compared to the obese MetS group (Tremblay-Franco *et al.*, 2015). The AA results of the current study were not statistically significant in any of the other subgroups (Figure 3.12B). Studies on the association between AA and body weight are inconsistent, since some studies observed unfavourable effects, while others did not detect any significant differences (Dunbar *et al.*, 2014, Fekete *et al.*, 2015; Tremblay-Franco *et al.*, 2015). The lack of significant differences may be attributed to the increased synthesis of pro-inflammatory eicosanoids, because obesity is associated with chronic low-grade inflammation, and increased utilisation of AA (Fekete *et al.*, 2015; Dugas *et al.*, 2016). Additionally, the association between endogenous FA synthesis and dietary FA intake may also have played a role (Spector, 2000; DeFilippis & Sperling, 2006; Fekete *et al.*, 2015).

4.4.5.5 The omega-3 to omega-6 ratio

An earlier study by Nigam *et al.* (2009) found that the MetS group presented with a significantly lower Ω-3:Ω-6 compared to their non-MetS counterparts. Contrary to these findings, Vanhala *et al.* (2012) found a significantly higher Ω-3:Ω-6 in the MetS group. Although these two aforementioned studies show contrasting results, the results of the current study, as well as that of Sethom *et al.* (2011) did not find any statistically significant difference in Ω-3:Ω-6 of the MetS and non-MetS groups (Table 3.3). Several reasons may account for these discrepant findings. Firstly, the choice of FAs to include in the calculation of the total Ω-3 FAs, since different studies included different combinations of Ω-3 FAs (Murff *et al.*, 2011). Examples of the different combinations include: (i) α-LA, EPA, DPA, and DHA; (ii) EPA, DPA, and DHA; (iii) α-LA, EPA, and DHA or (iv) only EPA and DHA. Some studies omitted α-LA in the calculation because it is poorly converted to EPA in humans, while others, and the present study, only included EPA and DHA, since these Ω-3 FAs have been shown to have anti-tumour promoting properties (Bagga *et al.*, 2002; Murff *et al.*, 2011; Pouchieu *et al.*, 2014). Secondly, differences in dietary intake of the study populations of the abovementioned studies may also account for contrasting findings (Vanhala *et al.*, 2012). The findings for Ω-3:Ω-6 in the current study may be due to the low intake of fatty fish and fish oils with concomitant high intake of Ω-6 vegetable oils in both the MetS and non-MetS groups (Table 3.5). Thirdly, IR may also play a role because insulin-resistant individuals do not respond to insulin-stimulated LPL-mediated hydrolysis of TGs (Vanhala *et al.*, 2012). Kabagambe *et al.* (2008) reported positive associations between insulin, HOMA-IR index and Ω-6 FAs. In the current study, the MetS group presented with significantly

higher insulin levels, which may have influenced Ω-6 FA levels, and it is possible that this played a role in the findings for the Ω-3:Ω-6 (Table 3.1 & Appendix K). The Ω-6 and -3 FAs have pro- and anti-inflammatory effects, which makes the Ω-3:Ω-6 clinically important, since its constituents compete for the same elongase and desaturase enzymes in different metabolic pathways (DeFilippis & Sperling, 2006; Odegaard, 2009; Murff *et al.*, 2011; Strandvik, 2011; Cottet *et al.*, 2015).

Sethom *et al.* (2011) found that obesity is associated with reduced FA oxidation that cause reduced PUFA levels. Since no significant differences were evident for LA and AA levels, the current study also did not observe significant differences for the Ω-3:Ω-6 of the respective groups (Figure 3.12C). No significant correlations were also noted between the Ω-3:Ω-6 and BMI for any of the groups (Appendix J, Table J.6). Arachidonic acid competes with EPA and DHA for enzymes and space in the cell membrane (Hussein, 2013) and this interaction between these FAs may explain the findings of the current study.

4.4.5.6 Additional factors influencing fatty acid levels

Fatty acid assessment method

Different studies used different methods to detect FAs in biological samples, and this complicates the comparison of the current results to that obtained from other studies, since the different tissues reflect different metabolic changes that influence FA levels (Baylin *et al*., 2005). Fatty acid levels in plasma or serum reflect dietary intake of the past few days, and may be compared with dietary assessment, since both reflect short-term dietary intake. The RBC membrane FAs reflect medium-term dietary intake changes that accumulate during the lifetime of the RBCs (± 120 days) (Jackson *et al*., 1997; Vessby *et al*., 2002; Hodson *et al.,* 2008; Patel *et al.,* 2010; Serra-Majem *et al*., 2012). The current study used the RBC membrane FA method to study FA composition, and this may have contributed to our findings.

Studies by Nigam *et al.* (2009) and Žák *et al.* (2014) illustrates the effect of FA assessment methods and reported no significant differences in the plasma α-LA in the MetS and non-MetS groups, which is in agreement with the results of the current study (Table 3.3). Even though the studies by Nigam *et al.* (2009) and Žák *et al.* (2014) used plasma samples, which reflect short-term FA levels and the present study used the RBC membrane FA method, which is indicative of medium-term FA levels (Jackson *et al.*, 1997; Patel *et al.*, 2010; Serra-Majem *et al.*, 2012) similar results were obtained. This could therefore suggest that plasma FA levels are comparable to RBC membrane FA composition.

Gender

Mayneris-Perxachs *et al*. (2014) reported that higher levels of PA and desaturase activities were more strongly related to an increased risk of the MetS in women, thereby suggesting that the MetS has a greater impact in women. Oestrogen upregulates desaturase activities, which explains these gender-related differences in FA levels (Hodson *et al*., 2008; Mayneris-Perxachs *et al*., 2014). Sexual dimorphism in lipid metabolism may explain the significantly higher Ω-3 FA levels and higher Ω-3 index, since studies observed that women have higher proportions of DHA, since oestrogen upregulates the conversion of α-LA to DHA *via* the Δ-6 desaturase, thereby increasing DHA biosynthesis (Hodson *et al*., 2008; von Schaky, 2010; Harris *et al*., 2012). Although studies suggest that FA levels are higher in women, and the current study only included women, these participants were selected regardless of menopausal status, and the age-associated decline in oestrogen may have affected the findings.

Physical activity

Lower physical activity and adiposity are reportedly associated with higher PLA levels, suggesting that energy imbalance affects FA levels (Mozaffarian *et al*., 2010). Warensjö *et al*. (2005, 2006) found no associations between physical activity and FAs, or SCD activity. In contrast, Vinknes *et al*. (2013) reported weak associations between physical activity and SCD activity, and suggested that physical activity has tissue-specific effects on SCD activity. These findings suggest that physical activity may have led to the non-significant findings of the current study, since fewer MetS women participated in moderate- (50.0 % *vs* 55.6 %), and vigorous-intensity (17.7 % *vs* 20.0 %) work compared to the non-MetS women (Appendix I.2). This was also the case for vigorous-intensity recreational activities (5.9 % of MetS and 10.9 % of non-MetS women), while a greater proportion of MetS women (76.5 % for MetS and 69.6 % for non-MetS) participated in moderate-intensity recreational activities.

Smoking status

Findings also suggest that smoking may have significant effects on the FA composition, since smokers presented with higher MUFA levels compared to non-smokers, possibly due to alterations in Δ-9 SCD activity or MUFA catabolism (Warensjö *et al*., 2005; Hodson *et al*., 2008). Likewise, Warensjö *et al*. (2006) also reported positive associations between smoking and PLA, OA, and desaturase activity. Smoking remains a prominent risk factor for obesity, IR, and CVD, since nicotine has unfavourable effects on glucose metabolism, and these metabolic alterations may affect FA composition (Warensjö *et al*., 2006). The majority of women in the current study in both the MetS (50.0 %) and non-MetS (45.7 %) groups were non-smokers, and no significant associations were observed between MUFAs (PA and OA levels) and smoking status in this population (Appendix I & L). A smaller proportion of women in the MetS group (35.3 %) were

current smokers compared to the non-MetS group (43.5 %). This suggests that smoking did not contribute to the findings for the different FAs and FA ratios of the current study; however, factors such as under-reporting and the lack of information on smoking duration, number of cigarettes smoked, and the effects of previous smoking may be confounding factors.

4.4.6 Biochemical blood analyses

4.4.6.1 Insulin-like growth factor-1

Although the link between elevated IGF-1 and cancer risk is well established, the association between IGF-1 and metabolic diseases is still questioned, since limited and contradictory data exists on the relationship between IGF-1 and the MetS (Boden, 2009; Doyle *et al.*, 2012; Ren & Anversa, 2015). The insulin/IGF-1 pathways have been associated with the MetS, since these pathways play a role in metabolic alterations.

Insulin-like growth factor-1 and obesity

Several studies reported significantly lower IGF-1 concentrations in women with the MetS (Parekh *et al.*, 2010; Friedrich *et al.*, 2013; Ren & Anversa, 2015), although the current study found significantly higher IGF-1 concentrations in the MetS women (Table 3.4). A possible explanation for this occurrence is that obesity is associated with IGF-1 concentration, since it stimulates adipocyte differentiation, which leads to increased adipocyte IGF-1 production and elevated free IGF-1 levels (Sierra-Johnson *et al.*, 2008; Faupel-Badger *et al.,* 2009; Donohoe *et al.*, 2012; Doyle *et al.*, 2012; Rajpathak *et al.*, 2012; Westley & May, 2013; Ren & Anversa, 2015).

The BMI of the MetS women of the current study was significantly higher than their non-MetS counterparts, suggesting that obesity may be responsible for their significantly higher IGF-1 concentration (Table 3.1). When BMI and the metabolic status were considered, the OBMetS+ group presented with a two and a half times higher IGF-1 concentration compared to OBMetS- women (Figure 3.15A). In addition, the OBMetS+ group also had significantly higher IGF-1 concentrations compared to the NMetS-. This was also apparent in the study by Rajpathak *et al.* (2012), where a positive association was established between free IGF-1 levels and BMI, and IGF-1 levels and WC. This is consistent with the findings of the current study where several measures of obesity (including BMI, WC, W:H, W:Ht, and SAD) and IGF-1 concentration were all significantly higher in the MetS compared to the non-MetS group (Table 3.1, 3.4 & Figure 3.7).

The current study also observed a significant strong positive association between IGF-1 and W:H for the OWMetS+ group (Appendix J, Table J.8). However, some studies found an inverse association between IGF-1 levels, BMI, and WC, and proposed that obesity is associated with weight-related changes in insulin and GH levels, and this causes the inverse relationship between IGF-1 levels and measures of adiposity (Parekh *et al.*, 2010). In agreement, the current study observed significant strong negative associations between IGF-1 concentrations and HC, and IGF-1 concentration and BMI for OWMetS+ women (Appendix J, Table J.8).

Insulin-like growth factor-1 and insulin resistance

Insulin resistance and elevated insulin levels promote GH receptor resistance, and this leads to decreased IGF-1 levels (Oh *et al.*, 2012). However, others suggest that IGF-1 also play a role in glucose homeostasis, since increased insulin levels are associated with the MetS. This can lead to decreased IGFBPs, thereby increasing the bioavailability of IGF-1 that could lead to elevated free IGF-1 levels (Sierra-Johnson *et al.*, 2008; Jeon *et al.*, 2011; Erasmus *et al.*, 2012; Klug *et al.*, 2012; Camargo *et al.*, 2014). Our findings are in agreement with the latter studies, since the MetS group presented with significantly higher insulin and IGF-1 concentrations (Table 3.1 & 3.4). However, insulin levels were only significantly higher in the OWMetS+ compared with the OWMetS- group of the current study (Appendix K). A proposed mechanism here is that hyperinsulinaemia and obesity increase GH expression, which stimulates IGF-1 production to increase IGF-1 levels (Westley & May, 2013). A second proposed mechanism is that the production of IGFBPs is downregulated to increase the bioavailability of IGF-1 (Westley & May, 2013; Ren & Anversa, 2015).

Confounding factors

A number of confounding factors may contribute to the contrasting results for IGF-1, including age, and ethnicity. Ageing was associated with reduced IGF-1 levels. Here, IGF-1 is involved in growth, and concentrations generally peak during puberty and decline progressively with ageing (Parekh *et al.*, 2010). Additionally, the association between age and IGF-1 is also related to the age-related decrease in GH, since GH stimulates IGF-1 production (Westley & May, 2013). However, the opposite was true for the current study – MetS women were significantly older (40.2 years *vs* 35.0 years), and yet MetS women presented with significantly higher IGF-1 concentrations than non-MetS women.

Ethnicity is another possible confounding factor, since previous studies have described ethnic differences in adipose tissue distribution, and prevalence of obesity (Faupel-Badger *et al.*, 2013;

Evans *et al.*, 2011). Obesity is associated with IGF-1 levels, which makes it possible that ethnicity may affect IGF-1 levels. In the same way, ethnicity may also have contributed to our findings, since women of different ethnic backgrounds were included.

In the current study, IGF-1 concentrations of some participants (n=5 for the MetS group and n=13 for the non-Mets group) were excluded, since values were either below the detection limit or extremely high, thus reducing the sample size, and this may have affected the statistical power of the results obtained. Possible reasons for the discrepant findings may also be the specificity of the ELISA kit used, the fact that different studies used different sample types to assess IGF-1 concentrations, and that different components of the IGF-1 axis were assessed (for example binding proteins and receptors) (Rajpathak *et al.*, 2012; Friedrich *et al.*, 2013; Levine & Levine, 2013; Westley & May, 2013).

4.4.6.2 Leptin

Leptin, the most abundant adipokine, has several functions relating to energy expenditure and -balance, satiety, and body weight (Braun *et al.*, 2011; Levine & Levine, 2013). Individuals with the MetS presented with significantly higher leptin concentrations compared to controls (Kawashima *et al.*, 2009; Esteghamati *et al.*, 2011; Chiu *et al.*, 2012; Abu-Farha *et al.*, 2014; Shah *et al.*, 2014; García-Jiménez *et al.*, 2015), which is in direct contrast to the findings of the current study (Table 3.4).

Leptin and the metabolic syndrome risk factors

Studies by Yun *et al.* (2011) and Esteghamati *et al.* (2011) suggested that leptin could be a significant predictor of the MetS, independent of BMI class. While leptin levels predicted MetS status and correlated with the number of individual MetS risk factors in women, these associations became insignificant after adjustment for BMI (Yun *et al.*; 2011; Esteghamati *et al.*; 2011). This is in agreement with the findings of the present study, since a significant difference was observed between leptin concentrations of the MetS and non-MetS groups, but not between the subgroups categorised according to MetS status and BMI (Table 3.4 & Figure 3.15B).

Several studies have also reported associations between leptin levels and BP, glucose and insulin levels, and HOMA-IR in persons with the MetS (Ganji *et al.*, 2009; Yun *et al.*, 2011; Chiu *et al.*, 2012; Cohen *et al.*, 2012; Shah *et al.*, 2012; Atanassova *et al.*, 2014; Abu-Farha *et al.*, 2014). Elevated leptin levels are associated with hypertension, since leptin increases arterial BP by stimulating the sympathetic nervous system, enhancing renal sodium resorption and by stimulating endothelin production, a vasoconstrictor (de Castro *et al.*, 2015). Several studies

suggested that the other individual components of the MetS might also contribute to the pro-inflammatory and IR state, including glucose, insulin and HOMA-IR (Ganji *et al.*, 2009; Lee *et al.*, 2009b; Bremer *et al.*, 2011). The association between leptin and glucose metabolism is explained by the leptin receptors located on the pancreatic β-cells to suppress insulin secretion (Chiu *et al.*, 2012). In the leptin-resistant state, leptin does not suppress glucose-stimulated insulin secretion and this leads to IR (Deng & Scherer, 2010; Esteghamati *et al.,* 2011; Chiu *et al.*, 2012). Leptin and obesity ultimately lead to a state of leptin resistance that increase the risk of several components of the MetS (Doyle *et al.*, 2012; García-Jiménez *et al.*, 2015; Mendonça *et al.*, 2015). All of these metabolic-associated parameters (SBP, DBP, and glucose and insulin levels) were significantly higher in the MetS women of the current study, but leptin levels were significantly lower (Table 3.1 & 3.4).

Leptin and obesity

Several MetS-associated factors influence leptin concentrations, including abdominal obesity, and insulin and glucose levels (Donohoe *et al.*, 2011; Doyle *et al.*, 2012). Obesity disrupts normal adipose tissue function, including the secretion of adipokines, such as leptin (Abu-Farha *et al.*, 2014). Leptin levels were also significantly higher in overweight compared to normal weight women without the MetS (Gómez-Ambrosi *et al.*, 2012). Increased adipose tissue mass, increased WC, W:H and/or BMI, and regional adiposity may influence circulating leptin levels, since leptin plays a role in appetite regulation, energy expenditure, and body weight (Li *et al.*, 2011b;Yun *et al.*, 2011; Chiu *et al.*, 2012; Shah *et al.*, 2014). Increased leptin levels in obese individuals is the result of both increased fat mass, and adipocyte hypertrophy that cause increased leptin secretion (Howard *et al.*, 2010). All measures of obesity were significantly higher in the MetS group of the current study, yet leptin concentrations were significantly lower (Table 3.1).

While many studies investigated the association between increased BMI and leptin levels, few have focused on the relationship between the MetS and leptin levels stratified according to BMI. Increasing leptin levels were associated with increasing BMI in participants with the MetS, because leptin levels are positively associated with body weight, and its levels were related to several measures of obesity, including increased WC, total adipose tissue mass, body fat, and regional adiposity (Yun *et al.*, 2011; Chiu *et al.*, 2012; Shah *et al.*, 2012; Abu-Farha *et al.*, 2014). Waist circumference is closely associated with the VAT (including abdominal fat), which is also regarded more pathogenic than SAT, because adipokine secretion is greater in the VAT compartment (Crowther & Norris, 2012; Willett & Hu, 2013). Despite increasing visceral adiposity (WC, W:H, SAD, and VAT:SAT) across groups categorised according to the MetS

status and BMI, no significant differences were observed for leptin concentration in the present study (Figure 3.15B).

Although we anticipated a positive relationship between WC and leptin concentrations, it was not observed (Appendix J, Table J.9). However, there was a very strong significant positive correlation between the SAD and leptin concentrations in the OBMetS- group (Appendix J, Table J.9), which is possible since the SAD reflects abdominal fat distribution, and visceral adiposity (Stewart & Sutton, 2012). We propose, as others did, that obesity might be associated with leptin resistance, which leads to impaired leptin- and insulin signalling (Esteghamati *et al.*, 2011; Pan *et al.*, 2014; Garcia-Jiménez *et al.*, 2015). This leads to a leptin deficient state, characterised by failed appetite regulation and energy expenditure, and increased body weight. In agreement, the current study observed that the participants in the OBMetS- subgroup had approximately two times higher leptin concentrations than those in the OBMetS+ subgroup (Figure 3.15B).

Leptin and obesity have also been implicated in the pathogenesis of lifestyle-associated cancers (Doyle *et al.*, 2012; García-Jiménez *et al.*, 2015; Mendonça *et al.*, 2015). The proposed mechanism is that the leptin-resistant state is associated with impaired leptin- and insulin signalling, and increased inflammation that contributes to IR and increased cancer risk, since obesity leads to macrophage activation, and this triggers cytokine overproduction that enhances inflammation (Chiu *et al.*, 2012; Doyle *et al.*, 2012; Barazzoni *et al.*, 2014; Atannasova *et al.*, 2014; Garcia-Jiménez *et al.*, 2015). It is proposed that the effect of leptin on intracellular pathways that control cell growth, apoptosis, and angiogenesis are involved in the pathogenesis of cancer (Rodríguez *et al.*, 2013; Mendonça *et al.*, 2015).

Confounding factors

Age, ethnicity, and lifestyle choices, were all confounding factors in the current study and may explain why our findings for leptin levels differ from previous studies. It is well known that with ageing, there is redistribution of adipose tissue from peripheral- to abdominal areas, and this may explain the association between decreasing leptin levels and ageing (Schautz *et al.*, 2012). This was also evident in the current study, where the significantly older MetS group also presented with significantly lower leptin levels (Table 3.1 & 3.4). Additionally, the age-related decline in oestrogen levels have also been implicated as responsible for the decrease in leptin levels that is associated with ageing (Marques-Vidal *et al.*, 2010; Schautz *et al.*, 2012). The current study did not classify participants according to menopausal status, and both pre- and

postmenopausal women were included, which could possibly explain the lack of significant findings.

Ethnicity may also have played a role, since Cohen *et al.* (2012) found that Black women presented with significantly higher leptin levels compared to their Caucasian counterparts, possibly due to differences in body fat distribution, and subsequent leptin production. The current study population included Caucasian, Coloured, and Black women, and although ethnic differences in leptin levels were not assessed, it might have played a role.

Zuo *et al.* (2013) found that leptin levels were mostly associated with a Westernised diet, while Jafari-Vayghan *et al.* (2015) failed to identify a relationship between major dietary patterns (including Western, healthy, and traditional), and leptin levels. Participants of the current study mainly consumed a Westernised diet, which may have affected leptin concentrations (Table 3.5 & Figure 3.16). Fasting state may also play a role in leptin levels, because short-term energy balance affects leptin release by adipocytes (de Castro *et al.*, 2015). Although most women of the current study were fasted, others were not, and this may have influenced the findings.

Smoking has also been shown to decrease leptin levels, and although the exact mechanism is still unclear, it is speculated that tobacco decreases basal metabolic rate, which is negatively associated with leptin levels (Marques-Vidal *et al.*, 2010; Cohen *et al.*, 2012). Lower leptin levels reported in smokers may also be due to increased leptin sensitivity among smokers, which also leads to lower body weight, and subsequent lower leptin release from adipocytes (Ganji *et al.*, 2009; Cohen *et al.*, 2012). The majority of women in both the MetS (50.0 %) and non-MetS (45.7 %) groups were non-smokers, followed by current smokers (35.3 % for the MetS group and 43.5 % for the non-MetS), which makes it unlikely that smoking played a role (Appendix I.1). However, smoking is a lifestyle factor that is often under-reported, which may have been the case here.

The specificity and sensitivity of the ELISA kit used to determine leptin concentrations might also explain the large variation in leptin concentrations of the present study. Additionally, the data for leptin concentration of several participants were excluded (n=six for the MetS group and n=14 for the non-MetS group) due to levels below the detection limit of the kit or levels that were too high (outliers) and this may have influenced the statistical power.

CHAPTER 5: CONCLUSION

5.1 INTRODUCTION

This chapter contains the main findings and conclusions of the current study, followed by the strengths and limitations of the data collection procedures. The chapter concludes with future recommendations.

The current study observed a MetS prevalence of 42.5 %, which is somewhat higher than prevalence rates reported for South-African populations. The majority of women in the MetS group presented with three MetS risk factors (58.8 %), while the majority of non-MetS women presented with two risk factors (54.3 %). It is also somewhat disturbing that only six women (13.0 %) in this population presented with no risk factors. Even more important is the fact that 20.6 % of women presented with four and five MetS risk factors, respectively. Although the predominant risk factors were similar in the MetS and non-MetS groups, the order in which they appeared differed for the two groups. Abdominal obesity was most predominant (100 %) in the MetS group, followed by hypertension (82.4 %), and low HDL-c (76.5 %). For the non-MetS group, hypertension was most prevalent (47.8 %), followed by abdominal obesity (39.1 %), and low HDL-c levels (34.8 %). The high prevalence of the MetS and its individual risk factors in women of this study are distubing, because the MetS is associated with an increased risk of developing T2DM, CVD, and lifestyle-associated cancers. Consequently, it is important for these women to be aware of these risks, and to make lifestyle changes to reduce their risk of developing these diseases later in life.

5.1.1 The influence of metabolic status

Our findings revealed the adverse effects of the MetS on physiological- and biochemical parameters, anthropometric measurements, and body composition. These findings suggest that obesity contributes to leptin resistance, and this leads to impaired leptin- and insulin signalling. Categorisation according to metabolic status did not indicate any significant differences for the individual FAs or FA ratios, which makes it difficult to draw an accurate conclusion linking the individual FAs or FA ratios to the MetS, and future studies are therefore needed to clarify this relationship. Metabolic status predicted changes in IGF-1 levels, thereby linking the MetS to increased cancer risk. This study also noted characteristic differences in the lifestyle behaviours of women with and without the MetS, and this may have played a role in our findings.

5.1.2 The combined influence of metabolic status and body mass index

The combined effect of the metabolic status and BMI only had an effect on some of the MetS risk factors (WC, SBP, DBP, and HDL-c levels), as well as IGF-1 levels. Although it also affected anthropometric (BMI, HC, W:H, and SAD) and BIA parameters (VAT and SAT areas, VAT % and SAT %, and VAT:SAT), it did not influence DBP, glucose, insulin, LDL-c, total cholesterol and leptin levels, or any of the individual FAs or FA ratios. The majority of women in the current study were either overweight or obese, and these findings confirm that adipose tissue dysfunction associated with these states contribute to the MetS. As a result, the classification of individuals according to metabolic status and BMI predicted various physiological-, anthropometric-, and body composition parameters, and indicated a possible effect on the parameters linking the MetS to cancer risk.

Strong significant associations were also evident between IGF-1 levels and the different measures of obesity, suggesting that obesity stimulates adipocyte IGF-1 production that leads to increased free IGF-1 levels. The current study showed significant correlations between measures of abdominal obesity (WC and SAD) and body composition (VAT %, SAT %, and VAT:SAT). Furthermore, VAT:SAT correlated with some metabolic parameters, including SBP, and TG and LDL-c levels, thereby supporting the notion that increasing VAT and abdominal obesity is associated with increased lipolysis, reduced insulin sensitivity, and impaired insulin signalling, which all increases the risk of the MetS, CVD, and lifestyle-associated cancers.

5.1.3 Correlation analyses

Correlation analyses indicated that obesity is a key determinant in the pathophysiology of the MetS, which was evident from significant associations between BMI and body composition (VAT %, SAT %, and VAT:SAT). These findings also confirm that anthropometric measures correlate with more direct measures of obesity. We also showed significant positive associations between SAT area and the Ω-3 index, and the Ω-3:Ω-6, possibly highlighting the anti-inflammatory properties of Ω-3 FAs on the obesity-associated chronic low-grade inflammation. However, the current study did not include inflammatory markers, thus no accurate conclusion could be drawn here.

5.2 STRENGTHS OF THE STUDY

A major strength of this study was that individuals without the MetS, and categorised according to BMI, were compared with individuals with the MetS, also categorised into the same BMI classes. The sample was ethnically diverse (Caucasian, Coloured, and Black participants) with a wide age range (18 to 60 years), BMI (18.6 to 53.8 kg/m^2), and WC (63.0 to 120.0 cm), enabling

comparisons with other study populations that made use of the same specifications. The current study investigated the pathophysiology of the MetS by including several factors, such as metabolic- and growth factors, additional measures of body fat distribution, and anthropometric measurements to assess body fat.

Students were trained in new techniques using standardised procedures and -measurements prior to starting the research project. According to our knowledge, this is a novel study, as it is the first time that the FA profile of farm workers in the Western Cape was investigated. Furthermore, the present study did not only determine individual FAs, but FA ratios and –metabolism were also investigated. Information regarding participant medication use and chronic diseases, lifestyle behaviours (including smoking and alcohol use), nutritional intake, and physical activity were available to the study, which enabled a more accurate and thorough interpretation of the results. While many other studies relied on a 24-hour recall or the lengthy FFQ, the present study obtained dietary intake data for three days, which could be used to estimate the usual dietary intake of participants.

5.3 LIMITATIONS OF THE CURRENT STUDY

This study made use of a cross-sectional study design, which is limited by the fact that causality cannot be established. Participants were conveniently recruited in the farming areas around Stellenbosch. Only female participants were included in the study, and the results obtained here can therefore not be generalised to the general farm worker population from this region. It is not unusual to encounter measurement errors during anthropometrical assessment such as positioning of the body, locating body landmarks in obese participants, and errors in reading measurements off equipment and recording it. Certain anthropometric measurements, including WC and HC, are dependent on the time of day, postural deviations, environmental temperatures, and lung function (depth of inspiration). Anthropometric measurements could not be validated and assessed using the gold standard, such as magnetic resonance imaging (MRI) and computed tomography (CT). The procedure to assess FA metabolism directly is complex, and therefore we could only use product-to-precursor ratios. Definitive conclusions about SCD enzyme activity could not be made here, although estimated enzyme activity is useful to interpret FA metabolism and desaturation patterns. Subjectivity and recall- and information bias are common problems when participants are asked to complete a three-day food record. Dietary intake may vary due to seasonality of certain products, and under- and over-reporting are significant limitations of this dietary assessment tool.

5.4 FUTURE RESEARCH AND RECOMMENDATIONS

Future research should use a randomised control study group consisting of men and women of different age- and ethnic groups in order to evaluate the gender-, age- and ethnic differences in the epidemiology of the MetS in the Western Cape. Additional surrogate measures for abdominal obesity (including the W:H and SAD) and fat distribution (VAT and SAT) may be useful in future MetS studies, since abdominal obesity is a critical component of the MetS. Inflammatory markers, such as IL-6, may be useful in future research, since obesity is associated with chronic low-grade inflammation.

It may also be useful to assess adiponectin levels, since this is an insulin sensitising adipokine associated with the MetS. Inclusion of these additional markers may improve our understanding of the effects of lifestyle factors and urbanisation on body composition, the constituents of the MetS, and the associated cancer risk. Future studies may also benefit from methods that are more sensitive to measure leptin and IGF-1 levels, since levels of some participants in the current study were excluded, as values were either too high or too low.

Findings for the RBC membrane FA analysis in the current study did not indicate any significant differences. Future studies may benefit from using plasma samples to determine FA levels complementary to dietary assessment, since both these methods reflect short-term intake and may be comparable (Vessby *et al.*, 2002; Hodson *et al.*, 2008; Patel *et al.*, 2010; Serra-Majem *et al.*, 2012). Intervention studies may also be useful to aid in our understanding of the FA profile of MetS participants. For example, Ω-3 FAs have beneficial effects on the components of the MetS, and for that reason, it may be useful to compare the metabolic-, inflammatory-, and FA profiles of non-MetS and MetS groups with and without Ω-3 supplementation.

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APPENDICES

Appendix A: Ethical clearance from Stellenbosch University

UNIVERSITEIT·STELLENBOSCH·UNIVERSITY
jou kennisvennoot · your knowledge partner

Ethics Letter

16-Oct-2014

Ethics Reference #: N13/04/052
Clinical Trial Reference #: N13/04/052
Title: Cancer risk during urbanisation: metabolic syndrome and cancer

Dear Doctor Theodore Nell,

The HREC approved your progress report dated 14 July 2014. The approval of this project has been extended for a further year.

Approval date: 03 September 2014

Expiry date: 03 September 2015

If you have any queries or need further assistance, please contact the HREC Office 0219389657.

Sincerely,

REC Coordinator
Franklin Weber
Health Research Ethics Committee 1

Appendix B: Consent form

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT:

Cancer risk during urbanisation: metabolic syndrome and cancer

REFERENCE NUMBER: N13/04/052

PRINCIPAL INVESTIGATOR: Dr Theo A. Nell

ADDRESS:

Department of Physiological Sciences

Mike de Vries Building

Room 2007

Stellenbosch University

CONTACT NUMBER: 021 8083147

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

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

What is this research study all about?

This study will only be done in Western Cape Health districts. We will need approximately 1000 patients. We are trying to gather information on laboratory tests, body composition and patient questionnaires profiles of people undergoing migration. By getting this information we would be able to assess the prevalence of the metabolic syndrome in different regions in the Western Cape and how this might increase risk of developing cancer.

More people are relocating to larger cities and with this, their traditional habits change. One factor that plays an important role is nutritional changes. There are also changes in your body where some might become overweight. Your participation will help us look at the markers that tell researchers what factors are important to look at. Blood will be taken by a registered medical nurse. It will then be sent away to PathCare (Stellenbosch) where metabolic-associated parameters will be measured. Other biochemical tests that will be done by PathCare include insulin, glucose, lipids, and hormone measurements. The remainder of your blood samples will be used to analyse omega-3 fats. The blood pressure and anthropometric evaluation, and life style questionnaire will be done at the clinic.

Why have you been invited to participate?

We are trying to gather information on people that live in certain areas in the Western Cape provincial health districts. Your participation will help us understand what factors could lead to the development of certain cancers if people migrate from rural to urban areas. There will also be questions asked about your diet at home and how active you are during the week. By donating blood to our study, you will be helping us to determine these profiles and how we can relate them to the current diagnostic tests to investigate the metabolic syndrome and development of cancer.

What will your responsibilities be?

We will need to examine you as one of the selected patients. A blood sample will then be taken for laboratory tests. There will be a lifestyle questionnaire that you need to complete with the help of the researcher. A registered anthropometrist, Dr Theo Nell, will also perform anthropometric measurements to measure your waist circumference, hip circumference, and arm circumference, the back of your arm's skin fold, height and weight. You will also be asked to lie on the examination bed where Dr Nell will use a special machine that will tell us how much fat is in your body. This will only take a few minutes and does not hurt you.

Will you benefit from taking part in this research?

Although there may not be any direct benefits to me/the participant by participating at this stage, future generations may benefit if the researchers succeed in finding out more about how migration could lead to increased number of people developing cancer. If you choose to know the results of your blood tests, we will make these available. However, you would have to discuss this information with your usual/personal doctor, in order to assess your medical status.

Are there any risks involved in your taking part in this research?

There are no more than minimal medical or physiological risks associated with this study.

I/the participant may feel some pain associated with having blood drawn from a vein in my arm, and may experience some discomfort, bruising and/or slight bleeding at the site. The anthropometrical test will require you to take some of your clothes and shoes off, but there is no pain involved during this procedure. All measurements will be done in private and confidentiality is very important. The machine that will be used to determine the fat in the body uses a very small electrical current that you will not feel.

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

It is your decision to participate or not and nothing will be done from the researchers' part or medical staff at the clinic/hospital to in any way to persuade you to take part.

Who will have access to your medical records?

Only the principal researcher (Dr Theo Nell and other collaborators) will have access to your data and records. All information will be treated with respect and utmost confidentiality. Under no circumstances will your name or any form of identification be used in any publication, poster, lecture or thesis that results from this study. Dr Theo Nell will be the only authorised personnel who will have access to all your results from this study as well as the lifestyle questionnaire and anthropometric measurements.

What will happen in the unlikely event of some form injury occurring as a direct result of your taking part in this research study?

There are no risks involved that could lead to injury. Not applicable here.

Will you be paid to take part in this study and are there any costs involved?

No, you will not be paid to take part in the study. There will be no costs involved for you, if you do take part.

Would you like to know the results of your blood tests?

Please indicate by marking the correct box with an X

YES ☐

NO ☐

Is there anything else that you should know or do?

You can contact Dr Theo Nell on 021 808 3147 if you have any further queries or encounter any problems. You can contact the Health Research Ethics Committee at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor. You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I ……..……. agree to take part in a research study entitled (Cancer risk during urbanisation: metabolic syndrome and cancer).
I declare that:

I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
I have had a chance to ask questions and all my questions have been adequately answered.
I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
I may be asked to leave the study before it has finished, if the study doctor or 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*) ……...…….. 2015.

…… ……

Signature of interpreter **Signature of witness**

Declaration by investigator

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

Signed at (*place*) .....…..…….. on (*date*) ……...…….. 2015.

…… ……

Signature of interpreter **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/Xhosa/Zulu/English.
- We encouraged him/her to ask questions and took adequate 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 his/her question satisfactorily answered.

Signed at (*place*) .....….....…….. on (*date*) ……...……2015

…… ……

Signature of interpreter **Signature of witness**

Appendix C: Fatty acid analysis protocol

SAMPLE COLLECTION AND PRE-TEST PREPARATION

- Blood samples for the FA profile were collected in two EDTA tubes, and spun down immediately (10 minutes @ 4000 rpm) to separate RBCs from plasma.
- The supernatant (plasma) was then drawn off and an equal volume of sterile 0.9 % saline solution at room temperature was added and mixed with the RBCs.
- The sample was centrifuged for four minutes @ 2500 rpm.
- The supernatant, as well as the WBCs were drawn off and discarded.
- This step was repeated three times until the supernatant was colourless, and clear and free of all WBCs.
- Following the final wash and complete removal of saline, the RBCs in the EDTA tubes were rapidly frozen on dry ice and stored at -80 °C until analysis.

FATTY ACID EXTRACTION

- Red blood cell samples in EDTA tubes were thawed at 4 °C overnight before the analysis.
- One mL methanol (CH_3OH) and 500 µL of RBC were placed in extraction tubes, and vortexed for 10 seconds.
- Two mL chloroform (CCl_3) was then added to each tube, and vortexed for 1 minute.
- After thorough extraction, 1.5 mL saline was added and the tube vortexed for 10 seconds.
- Tubes were then centrifuged for four minutes at 2000 rpm.
- The bottom chloroform phase with lipids were then transferred to transmethylation tubes.
- Tubes were then dried under a nitrogen stream (99.9 % nitrogen) in a waterbath set at 50-60 °C.

TRANSMETHYLATION OF FATTY ACIDS TO FATTY ACID METHYL ESTERS

- One hundred µL toluene and 2.0 mL methanol sulphuric acid (MeOH 5 % H_2SO_4) were added to each tube, vortexed briefly, and then incubated for two hours in a waterbath set at 70 °C.
- Two mL dH_2O and 2.0 mL hexane were added, and the tube vortexed for 20 seconds to extract FAMEs.
- The upper hexane layer was then transferred to a new transmethylation tube containing a small amount of sodium bicarbonate ($NaHCO_3$).
- Tubes were then dried under a nitrogen stream (99.9 % nitrogen) in a waterbath set at 50-60 °C.
- One hundred µL hexane was placed in each tube, and vortexed briefly.

- The contents of each tube were then placed in a GLC vial for FA analysis.

QUALITATIVE ANALYSIS

- Analysis was done by automated GLC (GLC, Thermo, Focus) equipped with flame ionisation detector.
- The peaks on the chromatograph were identified based on the retention time for each FA.
- Calculation of total FA and each FA expressed as a percentage of the total FAs.

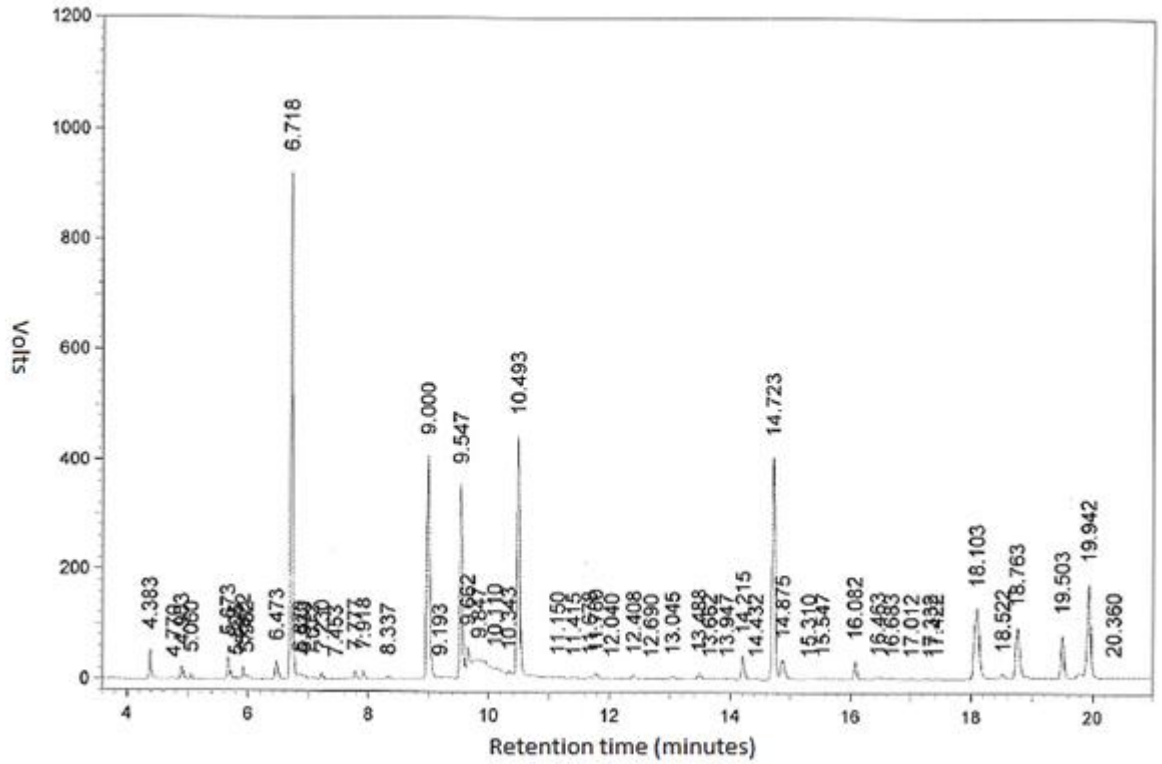


Figure C.1: Example of a gas chromatograph used to identify the different fatty acids based on retention times.

Table C.1. Retention times of different long chain fatty acids.

FATTY ACID	REFERENCE RANGE
14:0	5.070
14:1	5.400
16:0	6.728
16:1	7.242
18:0	9.020
18:1 n-9	9.568
18:1 n-11	9.683
18:2 n-6	10.510
18:3 n-6	11.163
18:3 n-3	11.695
20:0	11.812
20:1 n-9	12.437
20:2 n-6	13.517
20:3 n-6	14.237
20:4 n-6	14.742
20:3 n-3	14.815
22:0	14.905
22:1 n-9	15.577
20:5 n-3	16.105
22:2 n-6	16.718
24:0	18.137
22:4 n-6	18.545
24:1	18.797
22:5 n-3	19.523
22:6 n-3	19.967

Appendix D: Enzyme-linked immunosorbent assay (ELISAs)

Appendix D.1: Insulin-like growth factor-1 ELISA

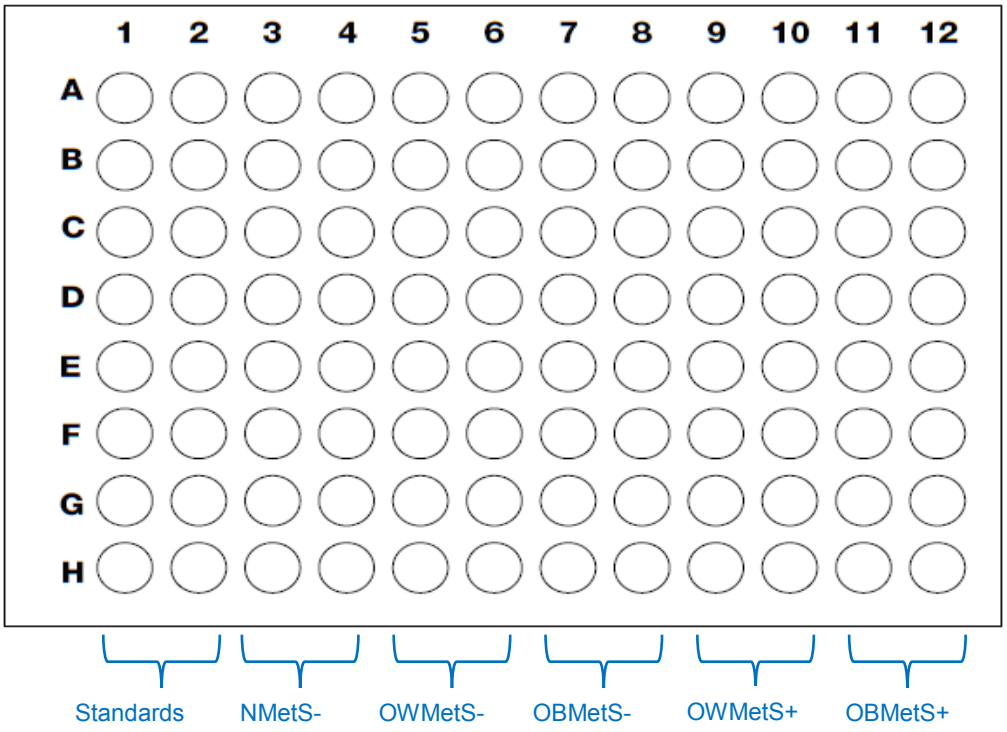
SAMPLE PREPARATION:

BODY MASS INDEX CLASS	DILUTION	VOLUME OF SAMPLE (µL)	VOLUME OF SAMPLE DILUENT (µL)	TOTAL VOLUME (µL)
Normal, overweight and obese	20x	12.50	237.50	250.00

(Adopted from: Yee *et al*., 2010; Bogin *et al*., 2015; Koeglenberg *et al*., 2015).

PLATE PREPARATION:

Each microplate contained the eight standards (columns 1 and 2) and eight samples of each of the different study groups (columns 3 to 12), as illustrated below. All samples were assayed in duplicate.



ASSAY PROCEDURE:

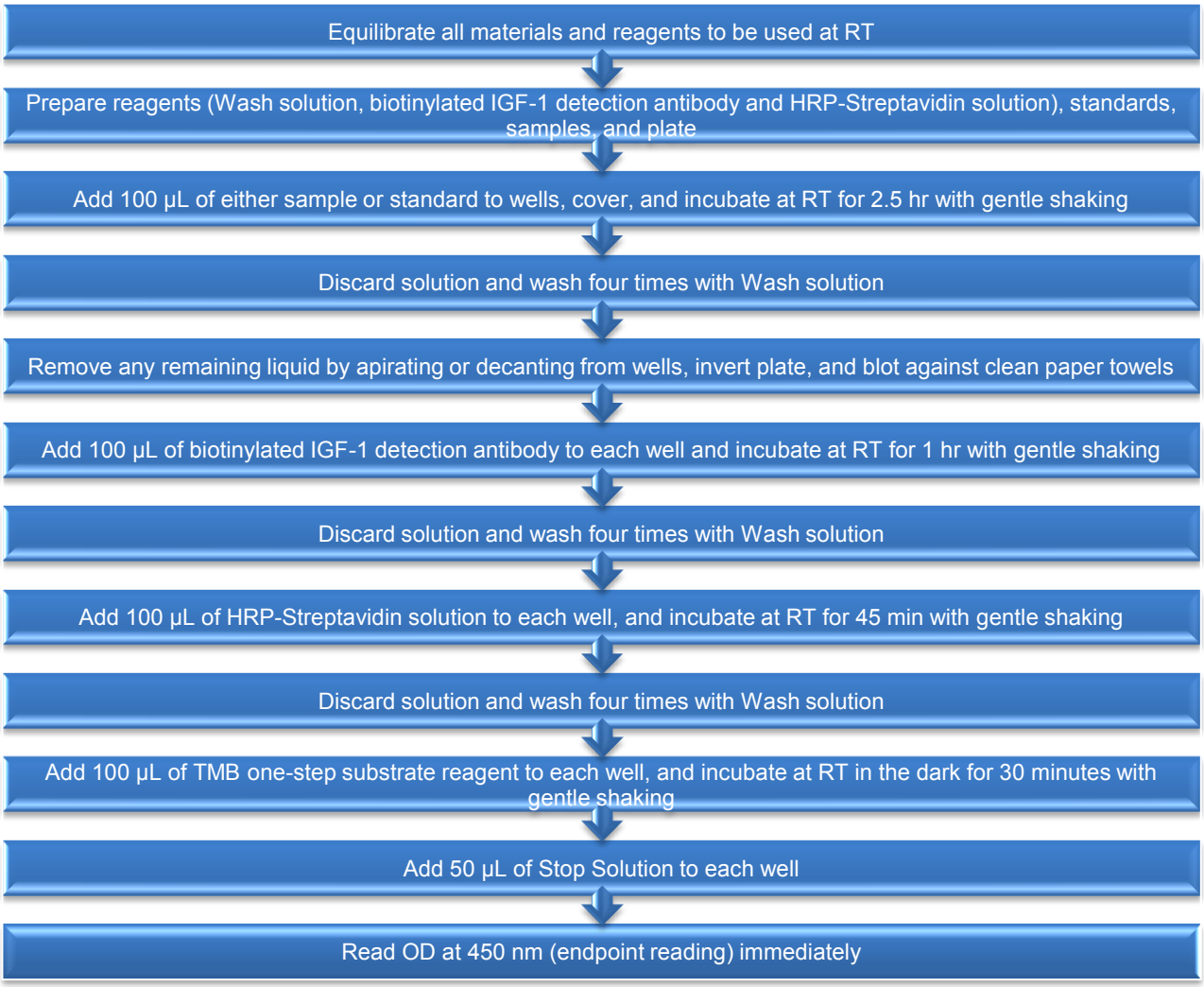


Table D.1. Method of measurements, detection limits, measureable concentration range and concentration of deficiency for haematology for insulin-like growth factor-1 levels of adults.

	IGF-1
Significance	Cancer risk through growth-promoting properties
Test used	Sandwich (quantitative) ELISA
Detection method	Colorimetric
Sample type	Serum/plasma
Storage of kit	-20 °C
Label or dye	HRP
Sensitivity	<0.20 ng/mL
Measureable concentration range	0.10-30.00 ng/mL
Dilution	20x
Assay duration	Multiple steps standard assay
Absorbance measured at	450 nm

Appendix D.2: Leptin ELISA

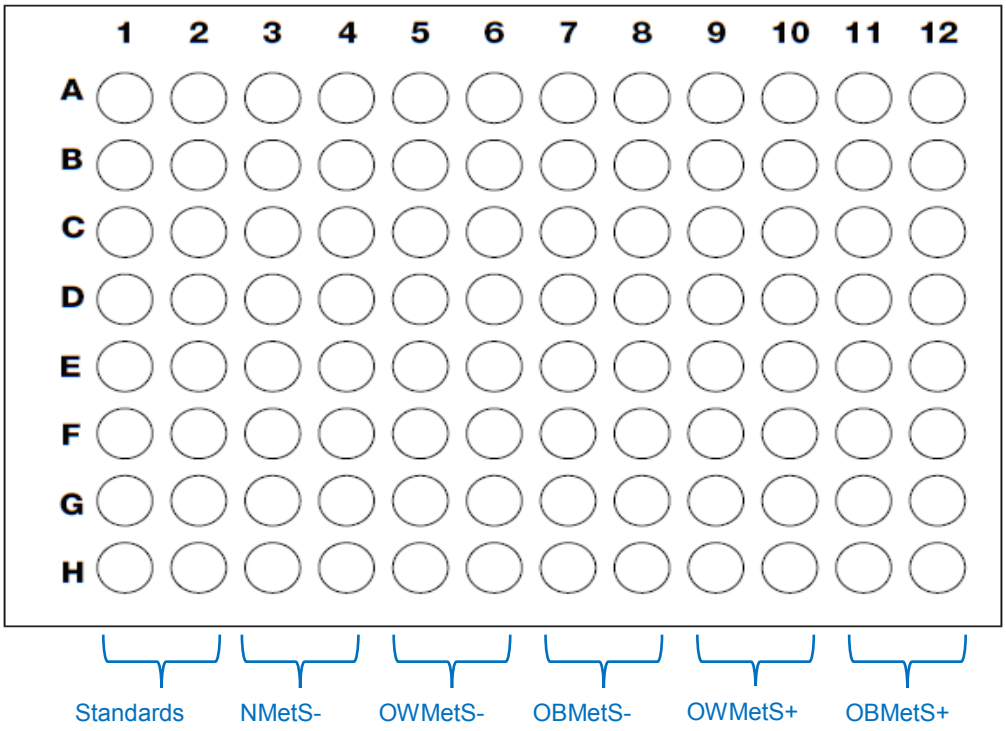
SAMPLE PREPARATION:

BODY MASS INDEX CLASS	DILUTION	VOLUME OF SAMPLE (µL)	VOLUME OF SAMPLE DILUENT (µL)	TOTAL VOLUME (µL)
Normal and overweight	40x	5.00	195.00	200.00
Obese	90x	4.40	395.00	399.40

(Adopted from: de Castro *et al*., 2015; García-Jiménez *et al*., 2015; Jafari-Vayghan *et al*., 2015).

PLATE PREPARATION:

Each microplate contained the eight standards and eight samples of each of the different study groups. All samples were assayed in duplicate as illustrated below.



ASSAY PROCEDURE:

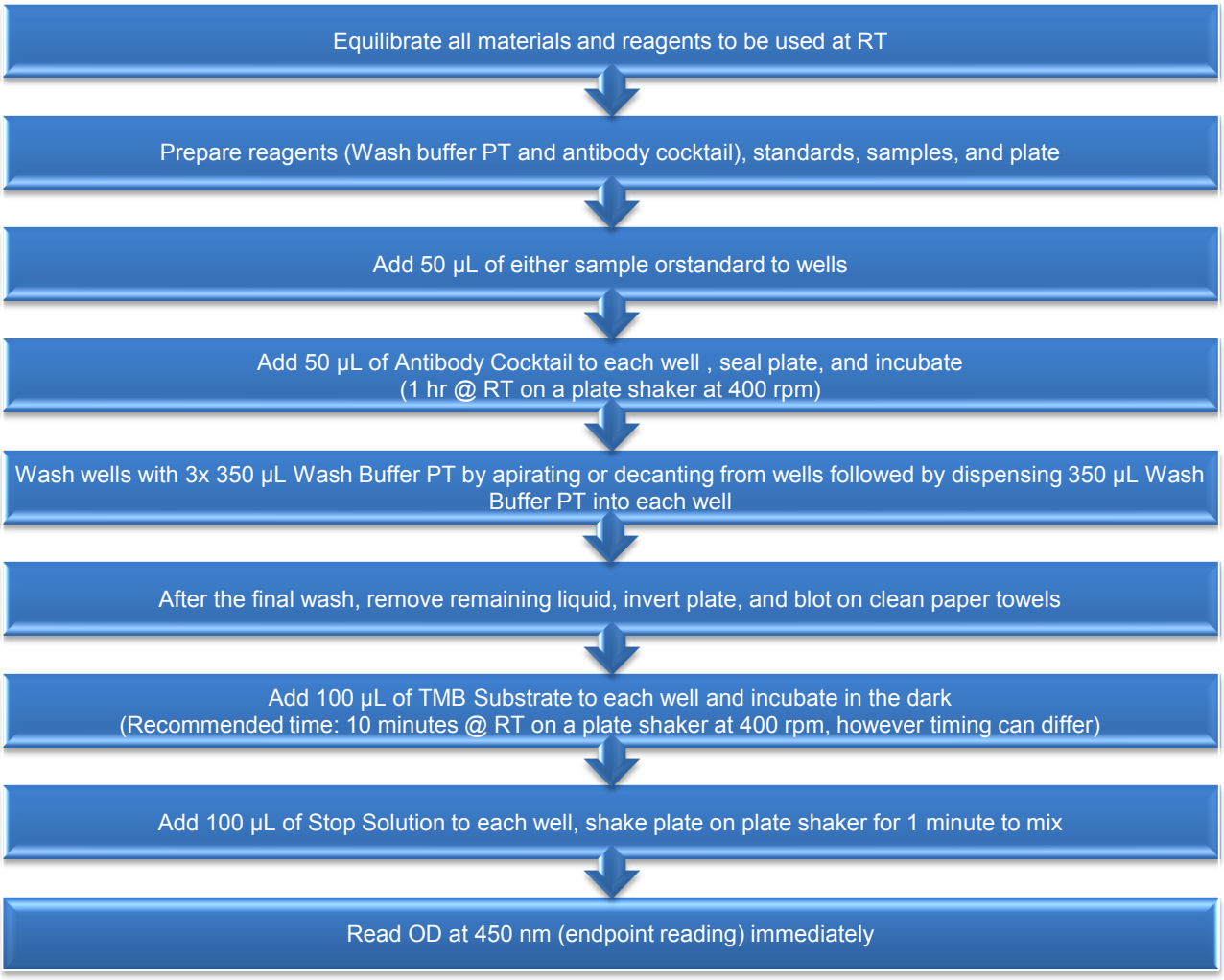


Table D.2. Method of measurements, detection limits, measureable concentration range and concentration of deficiency for haematology for leptin of adults.

	LEPTIN
Significance	Adipose tissue biomarker
Test used	Sandwich (quantitative) ELISA
Detection method	Colorimetric
Sample type	Serum/plasma
Storage of kit	4 °C
Label or dye	HRP
Sensitivity	5.30 pg/mL
Measureable concentration range	11.70-750.00 pg/mL
Dilution	20x for BMI = 18.50-29.99 kg/m^2 90x for BMI ≥30.00 kg/m^2
Assay duration	One step assay
Absorbance measured at	450 nm

Appendix E: Anthropometry data sheet

Id Code: _____

CANSA Study: Data Collection Sheet

Age	
Body Mass (kg)	
Height (m)	
BMI (kg/m^2)	
BP (mmHg)	
Heart Rate (b/m)	
WC (cm)	
HC (cm)	
W:H	
W:Ht	
SAD (cm)	
VAT	
Impedance (Ohms)	
Phase Angle	
SAT	
Impedance (Ohms)	
Phase Angle	

Appendix F: Bioelectrical impedance analysis protocol

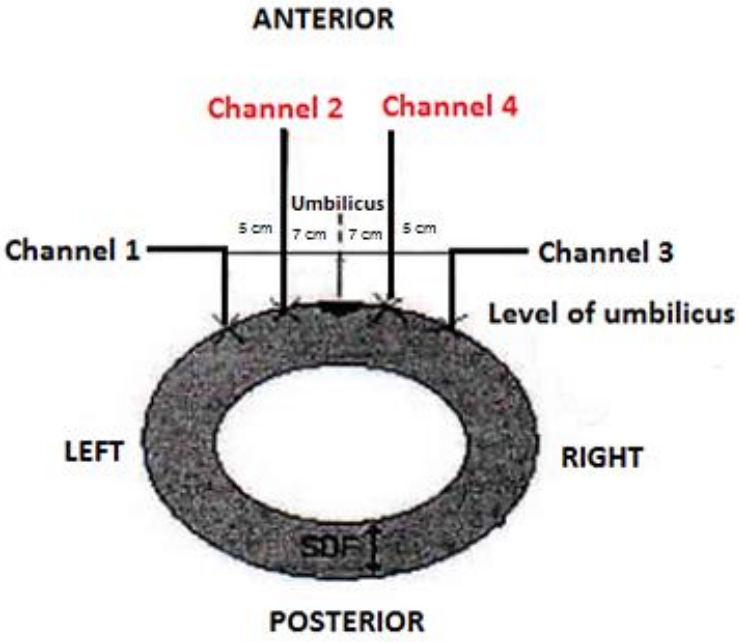
(Maltron BioScan 920II, 2015b).

MALTRON BioScan

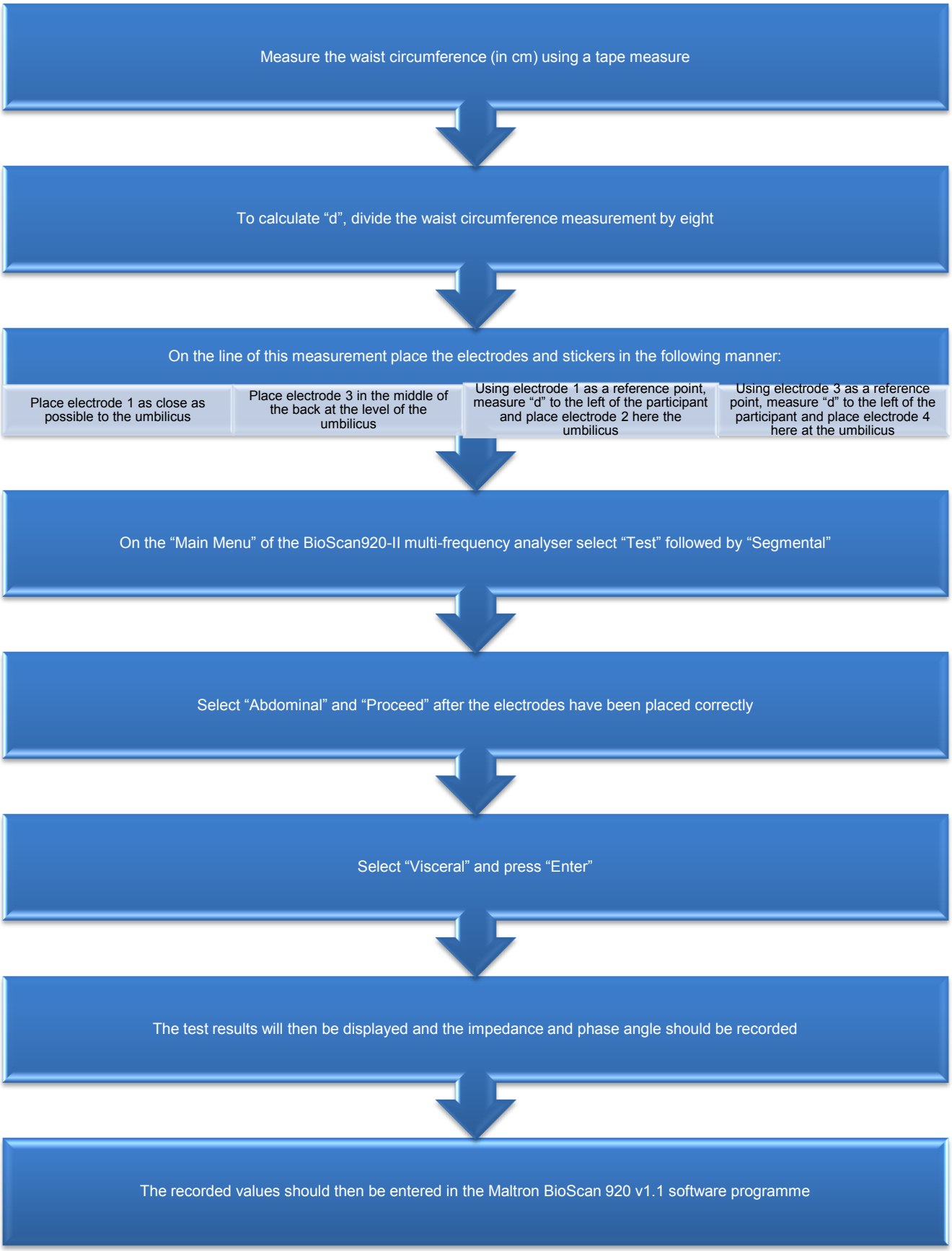
PRE-TEST PROTOCOL

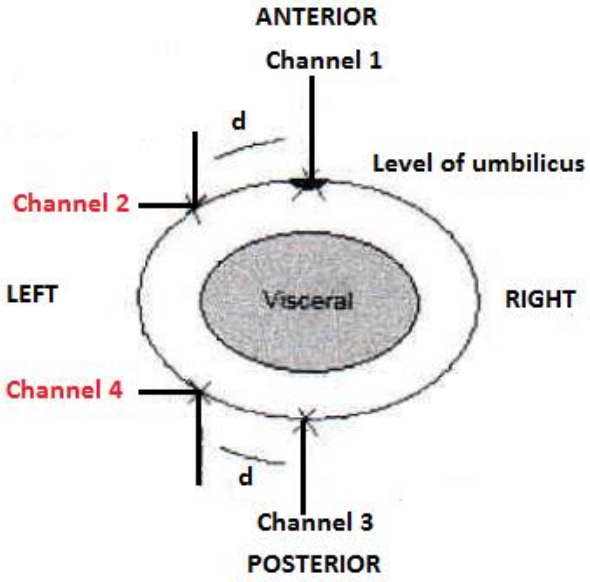
- Start by showing the participant the BioScan920-II multi-frequency analyser (Maltron BioScan 920II, United Kingdom) testing device and explain clearly, where you will place the electrodes and what you will be doing.
- Instruct the participant to remove any jewellery, metal objects, cell phones or other electronic equipment before the analysis started, since these may interfere with the conductivity and ultimately the readings on the machine.
- Participants were also asked to empty their bladders prior to testing because a full bladder may affect values for total body water.
- Participant stands in the upright position with feet apart.
- Areas on the abdomen where the electrodes will be placed are cleaned with alcohol swabs.
- The participant should refrain from alcohol intake for 12 hours prior to the procedure.
- The participant should not be diaphoretic (covered with sweat) or soaked in urine since the analyser measures this fluid as fat-free mass.
- The participant should not have exercised or taken a sauna within 8 hours of the procedure.
- Make sure analyser battery is relatively new and well charged.
- Regularly check analyser calibration and patient cables using a standard protocol.
- Turn the analyser on and make sure the subject refrains from movement.
- To ensure consistency and repeatability, electrodes should be placed exactly as shown in the illustrations. Variation in the placement of these electrodes will result in errors in the data.

ABDOMINAL SUBCUTANEOUS MEASUREMENT



ABDOMINAL VISCERAL MEASUREMENT





Appendix G: Three-day food record

RECORD KEEPING PERIOD

PERIOD	TIME FRAME	RECORD KEEPING WEEKS	RECORD KEEPING DAYS* - 3 CONSECUTIVE DAYS

* Day: Starts midnight and ends following midnight

Record keeping instructions

Record:

- Everything consumed – eat and drink
- Immediately – ensures nothing is forgotten/Less work
- Each day separate page – 2 pages per day (+ extra)
- Each food item new line
- Each food item detailed and accurately as possible

Information:

- *Column 1*: Time you ate/drank something, e.g. 13:00 (or 1 pm)
- *Column 2*: What you consumed, e.g. green beans, Rooibos tea
- *Column 3*: How the food was prepared, e.g. boiled, fried, sweetened
- *Column 4*: How much you consumed, e.g. ½ cup; 1 H tablespoon

HOW MUCH YOU CONSUMED

Last column: Quantity recordings

- ***Ruler***
  - Measure size of food about to eat
  - Length + Width + Height (Thickness) – Bread slice; Meat portion/Cold cut (Edible portion/Bone); Wedge/Slice
  - Height + Diameter – Fruit
- ***Measuring cup***
  - 500 mL capacity – Larger amount:
  - Measure volume about to eat/drink – Vegetable portions; Fluids
- ***Measuring spoons***
  - Teaspoon = 5 mL; Tablespoon = 15 mL; Also: Dessertspoon = 12.5 mL)
  - Smaller amount: Measure volume about to eat – Margarine/Butter as spread/Portions “dished up”

NOTE: Indicate *heaped (H)* or *level (L)*

- ***Volume/Weight***:
  - If known can be recorded – 175 mL yoghurt (container)

DO NOT ESTIMATE OR GUESS

Weighed with reliable scale

Similar/Standard amount consumed from day-to-day:

Measure precisely on first use (once) and record thereafter

WHAT YOU CONSUMED AND HOW IT WAS PREPARED

- Columns 2 and 3:

Describe extensively – Brand names/Attach recipes (mixed ingredients/dishes ≈ mixed vegetables; mixed herbs)

Cooking methods:

Boiling	Cooking (rapidly) in boiling water
Steaming	Cooking over boiling water in steam from water or in steam from food without added liquid
Frying	Cooking in oil (specify shallow-fried or deep-fried)
Baking	Cooking in oven
Roasting	Cooking in oven with small amount of fat or oil

FOOD	DESCRIPTION – Type/Kind	PREPARATION – Recipe descriptions
Bread/rolls	Brown; White; Whole wheat Hotdog; Hamburger	Additions/Accompaniments: Spreads: Butter; Margarine (type – hard, soft, light); Peanut butter; Jam (type – smooth, pieces); Cheese (type) NOTE: Sandwich (2 bread slices)
Porridge (cooked) **Cereals (ready-to-eat breakfast)**	Maize meal; Oats – traditional, instant Brand names – Flavour	Maize meal – soft cooked / stiff Additions / Accompaniments: Milk (type – full cream, low fat (2 %), fat-free (skim)) Sugar (type – white, brown) Honey; Sweetener (type) Butter; Margarine (type)
Rice	Brown; White	Cooked; Fried; Savoury
Pasta	Macaroni; Spaghetti	Sauce
Legumes	Lentils; Dried beans – Broad beans, Kidney beans, *etc*.	
Meat	Beef; Mutton; Pork Minced meat – Hamburger patties; Meatballs Cold cuts – Ham; Polony (French; Garlic), Sausages – Boerewors; Pork; Viennas; Frankfurters, Biltong; Dried sausage – Beef; Ostrich; Game - Lean / Fatty (visible fat) cuts	Fried (fat/oil and shallow-fried/deep-fried); Grilled; Roasted Stews; Casseroles Additions such as potatoes and other vegetables Boerewors – thick, medium or thin Additions / Accompaniments: Sauces (gravy)
Chicken	Drumstick; Thigh – With / Without skin	Batter; Crumbs Fried; Grilled; Roasted stews; Casseroles Additions such as potatoes and other vegetables

FOOD	DESCRIPTION – Type/Kind	PREPARATION – Recipe descriptions
Fish: Fresh / frozen **Fish: Tinned**	Hake; Snoek Sardines; Tuna; Pilchards (in tomato sauce); Mackerel	Batter; Crumbs; Grilled; Fried Fish cakes; Fish fingers
Eggs		Boiled; Fried Scrambled; Omelette Additions / Accompaniments: Milk; Vegetables; Cheese
Milk	Fresh; Reconstituted milk powder - full cream, low fat (2 %), fat-free (skim) Creamers (Brand names)	
Cheese	Cheddar; Gouda; Cottage – smooth, chunky, flavoured	
Soup	Type - Vegetable; Legume; Meat addition	Thin (watery) / Thick Additions / Accompaniments: Croutons; Parsley
Vegetables	Type – Fresh; Frozen; Canned - Side dish / Main ingredient - Ingredient in mixed dishes	Peeled / Unpeeled Whole; Sliced; Diced Steamed; Boiled; Stir-fried; Baked; Mashed (potatoes) Additions / Accompaniments: Butter/Margarine; Sugar; Salad dressings; Mayonnaise - Types
Fruit	Type – Fresh; Canned; Dried	Fresh – With / Without peel Canned – Syrup / Juice packed
Coffee	Pure; Blend; Filter; Instant	Additions: Milk; Sugar; Honey; Sweetener – Specify OR black, no sugar
Tea	English; Rooibos; Green; Herbal (Specify)	
Milk drinks	Brand (Milo; Horlicks; Nesquick, *etc*.)	
Fruit juices **Dairy mixes** **Cold drinks**	Flavours; Pure; Blends; Brand Flavours – Brand (Tropica; Fiesta, *etc*.) Fizzy drinks; Cordials (Brand: Oros; Lecol, *etc*.)	Sweetened / Unsweetened
Alcoholic drinks	Wine – Red; White; Rosé Brandy; Whisky, Beer; Sherry	
Cake; **Biscuits (Cookies);** **Rusks;** **Tarts**	Chocolate, Lemon Creams; Romany Creams; Tennis biscuits, Buttermilk, Apple; Milk	With / Without filling / icing (butter) Homemade / Commercial Additions / Accompaniments: Cream; Ice cream (Full cream; Sorbet); Custard (Milk type)
Pudding; **Ice cream**	Instant – Butterscotch; Banana Baked – Bread; Malva	Ingredients – Recipes Additions / Accompaniments: Cream; Ice cream (Full cream; Sorbet); Custard (Milk

FOOD	DESCRIPTION – Type/Kind	PREPARATION – Recipe descriptions
		type); Sauces (Chocolate)
Sweets	Chocolate: Slab – Milk; White; Dark; Bars; Assorted centres Hard / Soft jelly type Dried fruit squares, sticks, rolls Marshmallow – Plain / Coconut covered Peanut brittle Toffees Fudge	
Salty snacks	Potato chips (crisps) NikNaks; Cheese curls Pretzels Nuts – Peanuts Salty biscuits – Brands: Salticrax; Tuc; Cream crackers; Provita	Baked; Fried <u>Additions / Accompaniments</u>: Margarine; Spread; Dip; Cheese
Condiments	Tomato sauce; Chutney; Mustard Mayonnaise Other sauces	Less oil – Trim

EXAMPLE OF A DIETARY RECORD

TIME	WHAT DID YOU EAT/DRINK?	HOW WAS IT PREPARED?	HOW MUCH?
Example			
7:00	*ProNutro*	*Thick (with milk and sugar)*	*1 cup*
	2 % low fat milk		*½ cup*
	Sugar		*2 heaped teaspoons*
	Banana	*Fresh*	*1 large*
11:30	*Sandwich prepared from:*		
	Whole wheat bread	*Toasted*	*2 slices (thick; 15x2x10 cm)*
	Margarine	*Flora light*	*2 teaspoons (thinly spread)*
	Cheddar cheese	*Grated*	*¼ cup*
	Tomato	*Raw*	*1 small*
	Lettuce	*Fresh*	*1 leaf*
	Ham	*Cooked*	*2 sandwich slices*
13:00	*Cooked meal:*		
	Pumpkin	*Cooked, sweetened and with margarine*	*½ cup*
	Beans	*Cooked with potato and margarine*	*¾ cup*
	Fish (hake)	*Battered*	*2 fillets (60 g each)*
	Green salad (lettuce, tomato, cucumber and onion)	*Salad dressing, regular*	*1 cup + 1 tablespoon dressing*
	Coffee	*With milk and sugar*	*1 mug (300 mL)*
	Full cream milk		*30 mL*
	Sugar		*2 teaspoons*

Id Code: _____

DIETARY RECORD: DAY 1-3

Date: _____

Day of the week: _____

TIME	WHAT DID YOU EAT/DRINK?	HOW WAS IT PREPARED?	HOW MUCH?

Appendix H: Questionnaires

Appendix H.1: Familial cancer history

FAMILY CANCER HISTORY				Id Code:_____
Immediate family cancer diagnosis?	Mother	Father	Brother	Sister
What type of cancer:				
When diagnosed:				
Current status of cancer:	Newly diagnosed	Living with cancer	Remission	Deceased
Treatment:	Chemotherapy	Radiotherapy	Combination	No treatment

Appendix H.2: Smoking/tobacco use and alcohol consumption

SMOKING/TOBACCO USE			Id Code: _____
Smoking or tobacco use:	Non-smoker	Previous smoker	Current smoker
ALCOHOL CONSUMPTION			
Alchol consumption:	Non-drinker	Previous drinker	Current drinker

Appendix H.3: Global physical activity questionnaire (GPAQ)

GLOBAL PHYSICAL ACTIVITY QUESTIONNAIRE (GPAQ)	Id Code: _____

Next, I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person.

Activity at work:

Think first about the time you spend doing work. Think of work as the things you have to do such as paid or unpaid word, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effect and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate effort and cause small increases in breathing and heart rate.

Questions		Response	Code
1.	**Does your work involve vigorous-intensity activity that causes large increases in breathing and heart rate for at least 10 minutes continuously?**	Yes 1 No 2 *If No, go to P4*	P1
2.	**In a typical week, on how many days do you do vigorous-intensity activities as part of your work?**	Number of days: _____	P2
3.	**How much time do you spend doing vigorous-intensity activities at work on a typical day?**	_____hours: _____ minutes	P3 (a-b)
4.	**Does your work involve moderate-intensity activity that causes large increases in breathing and heart rate for at least 10 minutes continuously?**	Yes 1 No 2 *If No, go to P7*	P4
5.	**In a typical week, on how many days do you do moderate-intensity activities as part of your work?**	Number of days: _____	P5
6.	**How much time do you spend doing moderate-intensity activities at work on a typical day?**	_____hours: _____ minutes	P6 (a-b)

Travel to and from places:

The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to places of worship.

7.	**Do you walk or use a bicycle for at least 10 minutes continuously to get to and from places?**	Yes 1 No 2 *If No, go to P10*	P7
8.	**In a typical week, on how many days do you walk or bicycle for at least 10 minutes to get to and from places?**	Number of days: _____	P8
9.	**How much time do you spend walking or bicycling for travel on a typical day?**	_____hours: _____minutes	P9 (a-b)
Recreational activities: The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure).			
10.	**Do you do any vigorous-intensity sports, fitness or recreational (leisure) activities that cause large increases in breathing and heart rate for at least 10 minutes continuously?**	Yes 1 No 2 *If No, go to P13*	P10
11.	**In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational (leisure) activities?**	Number of days: _____	P11
12.	**How much time do you spend doing vigorous-intensity sports, fitness or recreational (leisure) activities on a typical day?**	_____hours: _____minutes	P12 (a-b)
13.	**Do you do any moderate-intensity sport, fitness or recreational (leisure) activities that cause large increases in breathing and heart rate for at least 10 minutes continuously?**	Yes 1 No 2 *If No, go to P16*	P13
14.	**In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational (leisure) activities?**	Number of days: _____	P14
15.	**How much time do you spend doing moderate-intensity sports, fitness or recreational (leisure) activities on a typical day?**	_____hours: _____minutes	P15 (a-b)
Sedentary behaviour: The following question is about siting or reclining at work, at home, getting to and from places, or with friends including time spent sitting at a desk, sitting with friends, travelling in car, bus or train, reading, playing cards or watching television. This does not include time spent sleeping.			
16.	**How much time do you usually spent sitting or reclining on a typical day?**	_____hours: _____minutes	P16 (a-b)

(Adopted from: WHO, 2004b).

Appendix I: Lifestyle factors

Appendix I.1: Smoking/tobacco use and alcohol consumption

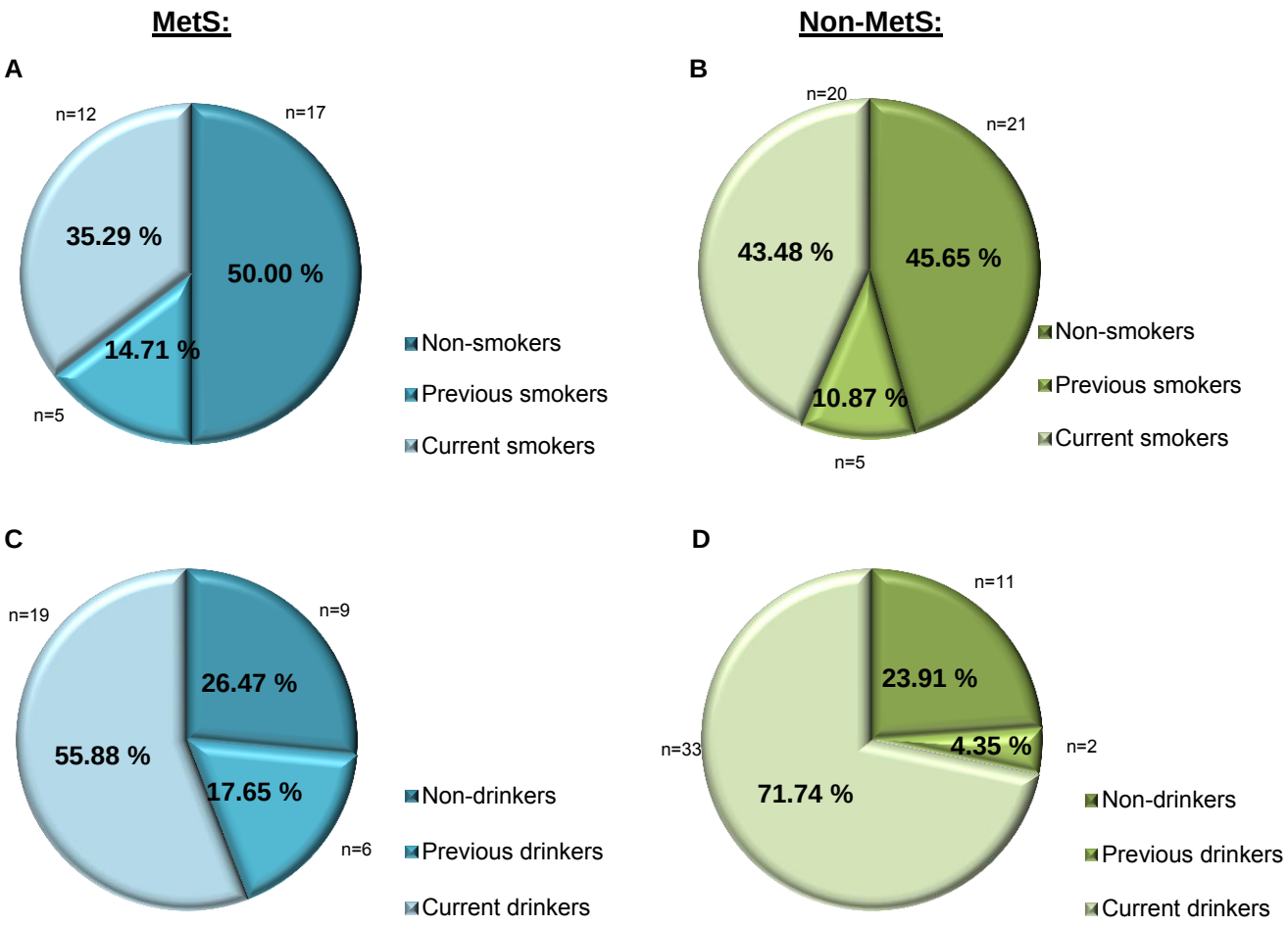


Figure I.1: The percentage of participants who are non-, previous- or current smokers and drinkers in the MetS (n=34) (A, C) and non-MetS (n=46) (B, D) groups.

Appendix I.2: Physical activity

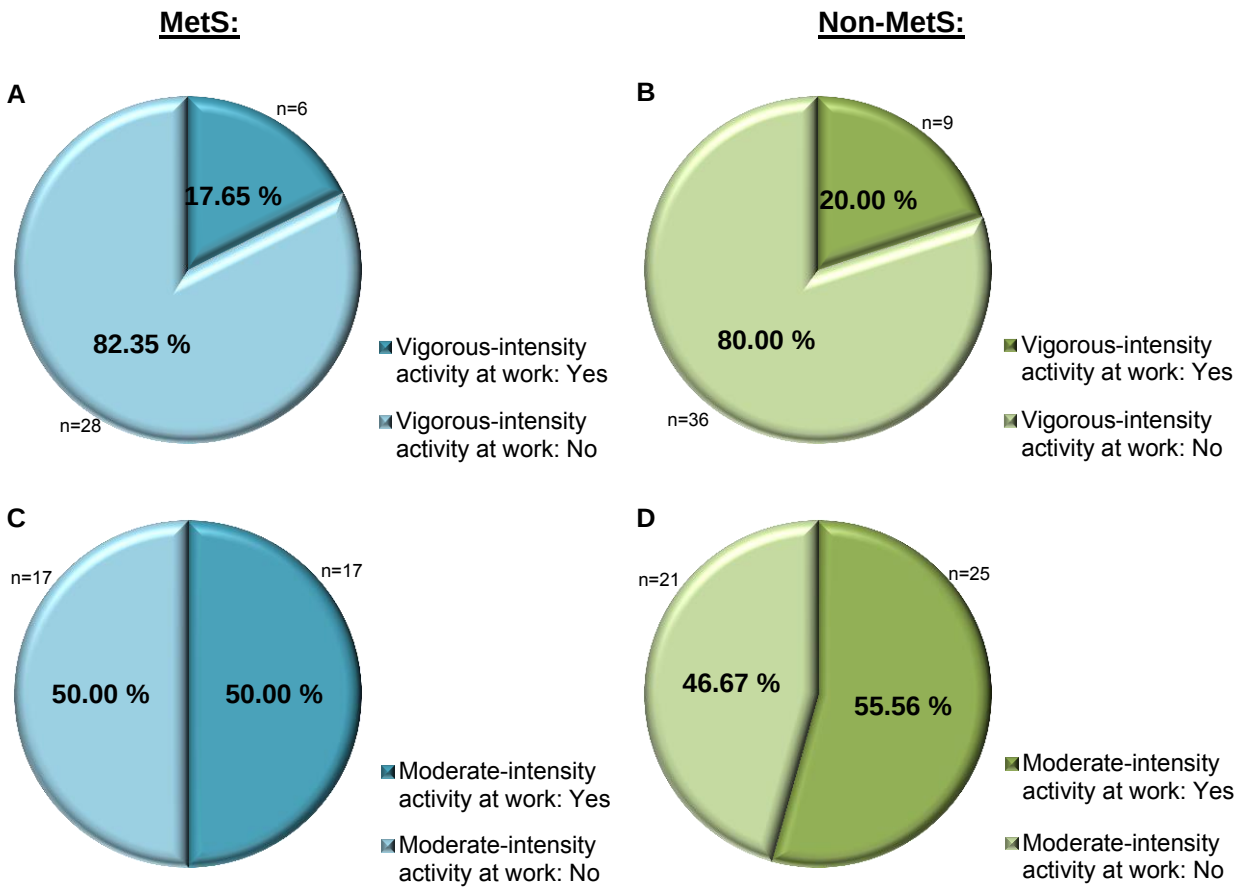


Figure I.2: The percentage of participants who partake in vigorous- and moderate-intensity physical activity at work in the MetS (n=34) (A, C) and non-MetS (n=46) (B, D) groups.

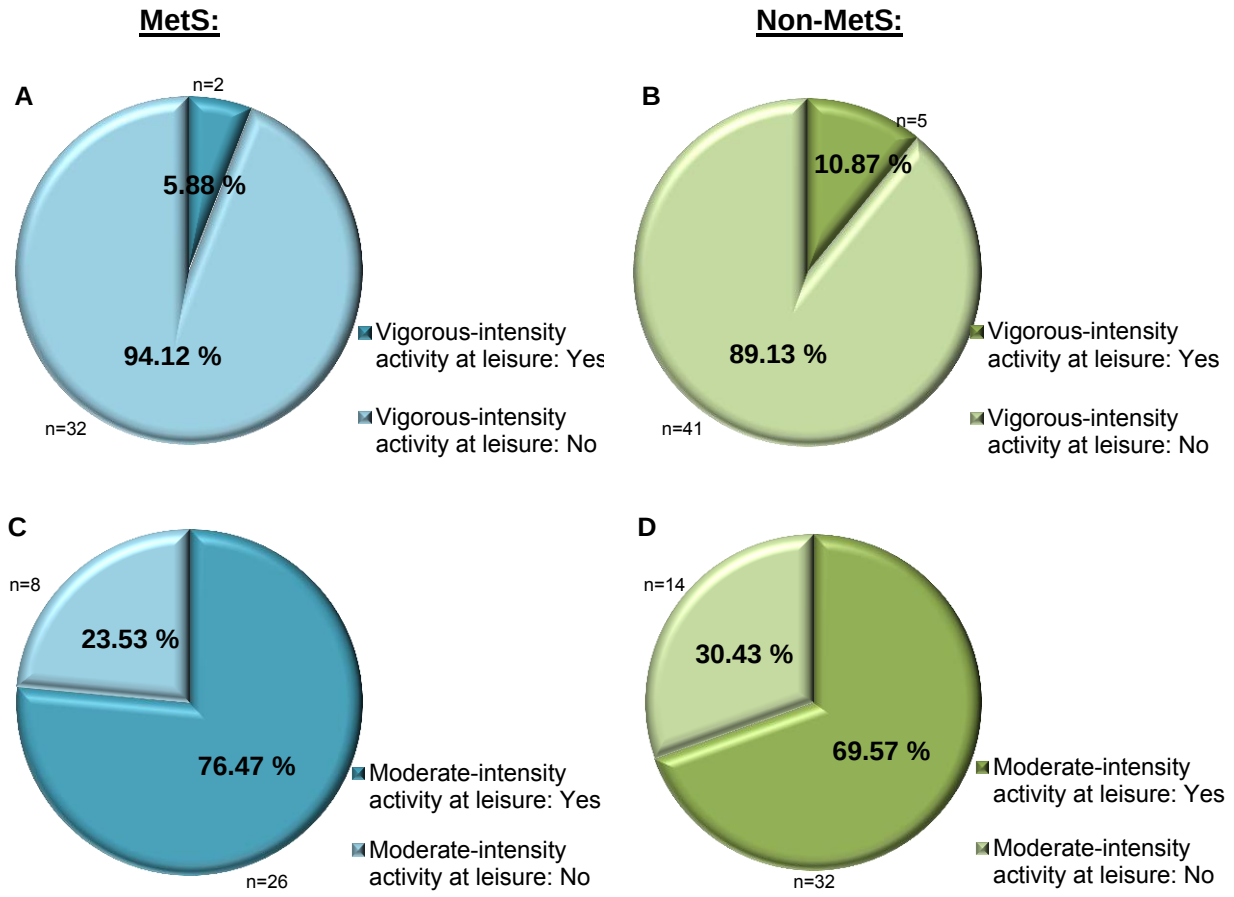


Figure I.3: The percentage of participants who partake in vigorous- and moderate-intensity physical activity during leisure in the MetS (n=34) (A, C) and non-MetS (n=46) (B, D) groups.

Appendix J: Supplementary correlation analyses

Table J.1: Relationship between waist circumference and the percentage visceral- and subcutaneous adipose tissue, and the VAT:SAT for the respective groups.

	WC & VAT %		WC & SAT %		WC & VAT:SAT	
	r-value	p-value	r-value	p-value	r-value	p-value
NMetS-	0.38	0.15	-0.38	0.15	0.42	0.11
OWMetS+	0.39	0.12	-0.39	0.12	0.34	0.18
OWMetS-	0.40	0.11	-0.40	0.11	0.41	0.10
OBMetS+	0.72	0.00	-0.72	0.00	0.74	0.00
OBMetS-	0.84	0.00	-0.84	0.00	0.84	0.00

Table J.2: Relationship between sagittal abdominal diameter and the percentage visceral- and subcutaneous adipose tissue, and the VAT:SAT for the respective groups.

	SAD & VAT %		SAD & SAT %		SAD & VAT:SAT	
	r-value	p-value	r-value	p-value	r-value	p-value
NMetS-	0.30	0.25	-0.30	0.25	0.27	0.31
OWMetS+	-0.07	0.79	0.07	0.79	-0.03	0.92
OWMetS-	-0.01	0.98	0.01	0.98	-0.02	0.93
OBMetS+	0.62	0.01	-0.62	0.01	0.63	0.01
OBMetS-	0.85	0.00	-0.85	0.00	0.81	0.00

Table J.3: Relationship between age and W:H for the respective groups.

	Age & W:H	
	r-value	p-value
NMetS-	0.50	0.05
OWMetS+	0.13	0.62
OWMetS-	0.28	0.28
OBMetS+	0.22	0.40
OBMetS-	-0.51	0.08

Table J.4: Relationship between VAT:SAT and some of the individual components of the metabolic syndrome for the respective groups.

	VAT:SAT & SBP		VAT:SAT & HDL-c		VAT:SAT & LDL-c	
	r-value	p-value	r-value	p-value	r-value	p-value
NMetS-	-0.14	0.61	-0.02	0.93	0.55	0.03
OWMetS+	-0.18	0.50	-0.00	1.00	0.48	0.05
OWMetS-	-0.10	0.71	0.49	0.048	0.47	0.06
OBMetS+	0.64	0.01	-0.25	0.34	-0.21	0.41
OBMetS-	0.11	0.72	0.43	0.14	0.06	0.84

Table J.5: Relationship between triglyceride levels and the percentage visceral- and subcutaneous adipose tissue, and the VAT:SAT for the respective groups.

	TG & VAT %		TG & SAT %		TG & VAT:SAT	
	r-value	p-value	r-value	p-value	r-value	p-value
NMetS-	0.47	0.07	-0.47	0.07	0.47	0.06
OWMetS+	0.27	0.29	0.27	0.29	0.21	0.42
OWMetS-	-0.13	0.63	0.13	0.63	-0.11	0.69
OBMetS+	0.18	0.49	-0.18	0.49	0.16	0.53
OBMetS-	0.03	0.93	-0.03	0.93	0.03	0.93

Table J.6: Relationship between W:H and palmitoleic acid levels, and body mass index and the omega-3:omega-6 for the respective groups.

	W:H & PLA		BMI & Ω-3:Ω-6	
	r-value	p-value	r-value	p-value
NMetS-	-0.02	0.95	-0.28	0.29
OWMetS+	-0.14	0.58	0.17	0.51
OWMetS-	0.27	0.29	0.00	0.99
OBMetS+	-0.13	0.63	-0.05	0.85
OBMetS-	-0.25	0.42	0.46	0.11

Table J.7: Relationship between percentage visceral adipose tissue and oleic- and arachidonic acid levels, the n-9 saturation index, and the delta-9 SCD 2 for the respective groups.

	VAT % & OA		VAT % & AA		VAT % & n-9 SI		VAT % & Δ-9 SCD 2	
	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value
NMetS-	0.14	0.59	0.03	0.92	-0.31	0.24	0.26	0.33
OWMetS+	0.14	0.60	-0.03	0.92	-0.18	0.48	0.27	0.30
OWMetS-	0.18	0.49	-0.31	0.23	-0.23	0.37	0.42	0.09
OBMetS+	0.29	0.26	0.12	0.65	-0.20	0.45	-0.04	0.88
OBMetS-	-0.23	0.46	-0.17	0.57	-0.00	1.00	-0.07	0.83

Table J.8: Relationship between insulin-like growth factor-1 concentration and measures of obesity for the respective groups.

	IGF-1 & W:H		IGF-1 & HC		IGF-1 & BMI	
	r-value	p-value	r-value	p-value	r-value	p-value
NMetS-	-0.23	0.43	-0.12	0.69	-0.22	0.45
OWMetS+	0.80	0.00	-0.69	0.00	-0.55	0.03
OWMetS-	0.35	0.27	-0.13	0.68	-0.07	0.83
OBMetS+	0.11	0.70	0.06	0.84	0.15	0.60
OBMetS-	0.31	0.45	-0.32	0.44	-0.17	0.70

Table J.9: Relationship between leptin concentration and waist circumference, and sagittal abdominal diameter for the respective groups.

	Leptin & WC		Leptin & SAD	
	r-value	p-value	r-value	p-value
NMetS-	0.23	0.55	0.14	0.72
OWMetS+	-0.17	0.54	-0.07	0.82
OWMetS-	0.05	0.86	0.10	0.76
OBMetS+	0.06	0.83	0.08	0.78
OBMetS-	0.49	0.15	0.74	0.01

Appendix K: Additional components of the metabolic syndrome

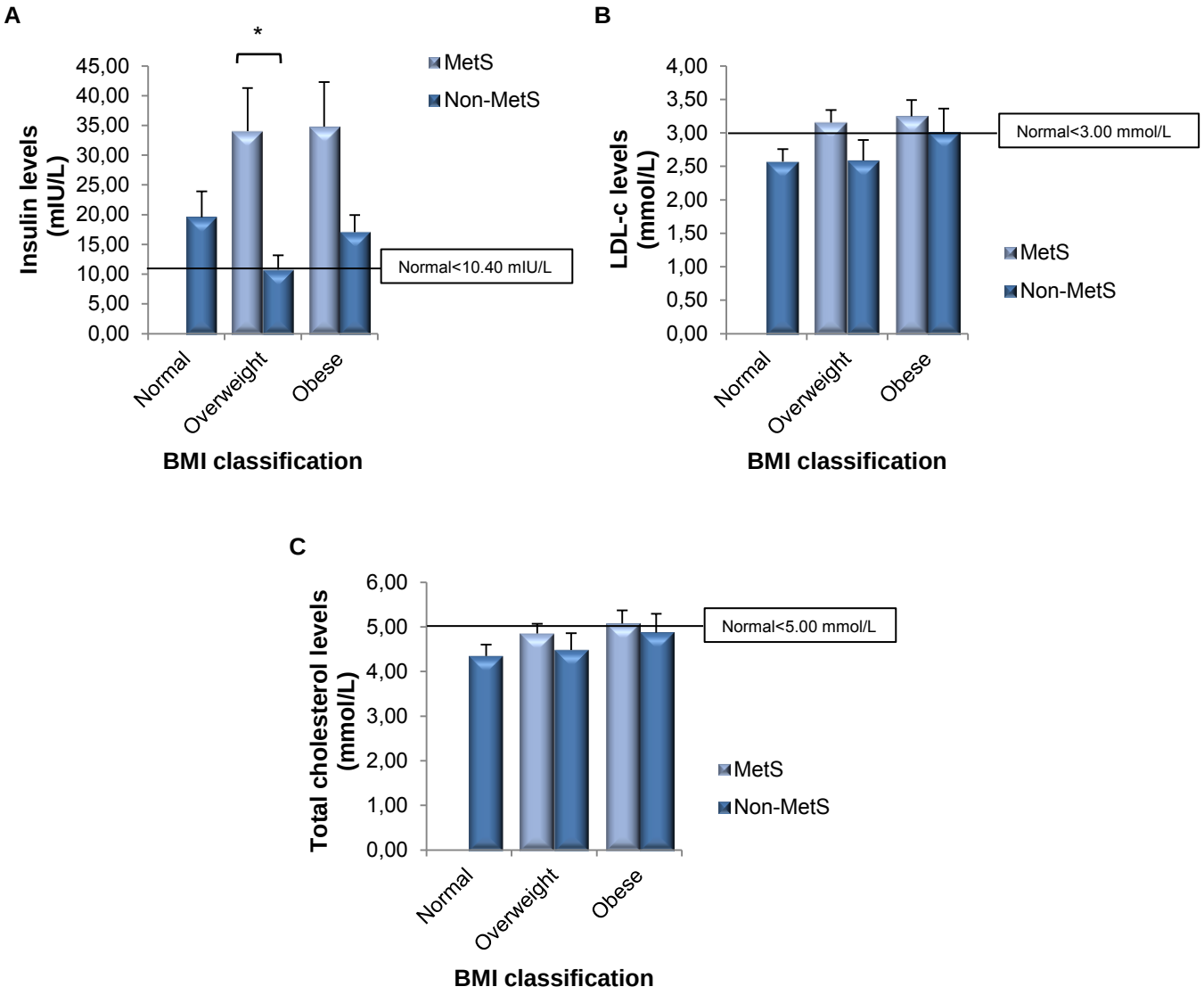


Figure K.1: Additional metabolic risk factors, (A) insulin, (B) low-density lipoprotein cholesterol, and (C) total cholesterol levels. Solid lines represent normal cut-off values.
(IDF, 2006; Klug *et al*., 2015).
* p<0.05, ** p<0.01, *** p<0.001.

Appendix L: Association between some individual monounsaturated fatty acids and smoking status for participants with and without the MetS

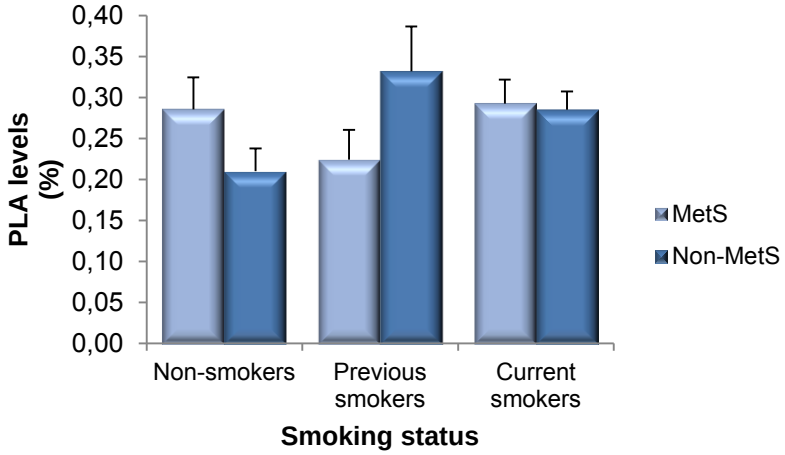


Figure L.1: Association between palmitoleic acid levels and smoking status for participants with (n=34•) and without (n=46‡) the MetS.

* p<0.05, ** p<0.01, *** p<0.001.

• Non-smokers (n= 17, 50.0 %), previous smokers (n= 5, 14.7 %) and current smokers (n=12, 35.3 %) for the MetS group.

‡ Non-smokers (n= 21, 45.7 %), previous smokers (n=5, 10.9 %) and current smokers (n=20, 43.5 %) for the non-MetS group.

Figure L.2: Association between oleic acid levels and smoking status for participants with (n=34•) and without (n=46‡) the MetS.

* p<0.05, ** p<0.01, *** p<0.001.

• Non-smokers (n= 17, 50.0 %), previous smokers (n= 5, 14.7 %) and current smokers (n=12, 35.3 %) for the MetS group.

‡ Non-smokers (n= 21, 45.7 %), previous smokers (n=5, 10.9 %) and current smokers (n=20, 43.5 %) for the non-MetS group.