Association between Cancer, Adipose Tissue and Selected Systemic Markers: a Possible Classification According to Body Shape

By

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DECLARATION

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ABSTRACT

Background: The metabolic syndrome (MetS) is a cluster of risk factors associated with an increased risk of developing chronic diseases of lifestyle, and has more recently been associated with cancer risk. Currently, the pathophysiology of the MetS and cancer risk is still unknown; however it is proposed to involve several factors. These include the effects of body composition (android and gynoid shapes), and insulin resistance on the bioavailability of growth factors, inflammatory markers and sex hormone profiles. Various anthropometrical measurements have been used to investigate body composition, however, due to their limitations, a new metric namely a body shape index (ABSI) has been proposed to be a better measure of fat distribution and body shape.

Aims: To determine the prevalence of the MetS, and the possible risks of developing cancer in relation to metabolic status, body composition, growth factors as well as inflammatory and sex hormone parameters.

Methods: Female participants between the ages of 20-60 years were classified according to the International Diabetes Federation's (IDF) definition of the MetS and according to body shape (android/gynoid) by photoscopic somatotyping. A series of tests and assessments were conducted; such as blood pressure assessments, anthropometric measurements, bioelectrical impedance analyses (BIA) and blood analyses. Blood analysis included fasting glucose, fasting insulin, lipid profile, insulin-like growth factor-1 (IGF-1), inflammatory marker (C-reactive protein (CRP)); and sex hormone parameters (oestrogen, female testosterone, sex hormone binding globulin; and free androgen index).

Results: The prevalence of the MetS was found to be 57.5 %; with abdominal obesity (73.8 %), elevated blood pressure (BP, 68.8 %) and low high density lipoprotein-cholesterol (HDL-c) levels (68.8 %) being the more prevalent risk factors. Both metabolic status; and body shape alone were found to be predictors influencing anthropometric, BIA, physiological and biochemical blood parameters. Metabolic status was found to have an effect on several parameters in the gynoid body shape groups, i.e. body mass (BM) ($p<0.001$), hip circumference (HC) ($p<0.01$), body mass index (BMI) ($p<0.001$), fat mass (FM) (%) ($p<0.01$), fat free mass (FFM) (%) ($p<0.01$), waist circumference (WC) ($p<0.001$), HDL-c ($p<0.001$), triglycerides (TG) ($p<0.05$), systolic blood pressure (SBP) ($p<0.05$) and diastolic blood pressure (DBP) ($p<0.01$), while metabolic status showed an effect on BM ($p<0.001$), BMI ($p<0.01$), TG ($p<0.05$), SBP ($p<0.01$) and DBP ($p<0.01$) in the android body shape groups. Both metabolic status and body shape did not show any effect on ABSI, total cholesterol (TC), low density lipoprotein-cholesterol (LDL-c), fasting insulin, CRP and all sex hormone parameters. Correlation analyses revealed significant correlations for several anthropometric, BIA and blood parameters.

Conclusion: This study showed that metabolic status, body shape and/or both could predict changes in various body composition, physiological and biochemical parameters in women. However, no effects were evident for any parameters linking the MetS to cancer risk. Thus, no accurate conclusion could be drawn regarding the pathophysiology. Our findings on ABSI, still warrants future investigation to substantiate the use of this metric in relation to the MetS, body shape and cancer risk.

Key words: Metabolic syndrome, body shape, cancer

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OPSOMMING

Agtergrond: Die metaboliese sindroom (MetS), wat bestaan uit ‘n groep risiko faktore wat met ’n toename in die ontwikkeling van chroniese lewenstyl siektes geassosieër word, is onlangs ook met kanker geassosieër. Tans, is die patofisiologie van die MetS en kanker risiko onbekend; hoewel dit moontlik verskeie faktore kan insluit. Hierdie faktore sluit onder andere in; liggaamsamestelling (androïede en genoïede vorme), asook insulienweerstandigheid en die biobeskikbaarheid van groei faktore, inflammatoriese merkers en geslagshormoon profiele. Verskeie antropometriese metings word gebruik om liggaamsamestelling te bepaal, maar as gevolg van beperkinge, word ’n nuwe maatstaf, naamlik die liggaamsvorm indeks (LVI) voorgestel as ’n beter meting om vet verspreiding en liggaamsvorm te beskryf.

Doelwitte: Om die voorkoms van die MetS, en die moontlike risiko vir die ontwikkeling van kanker wat verwant is aan die MetS, liggaamsamestelling, groei faktore, asook inflammatoriese en geslagshormoon parameters te bepaal.

Metodes: Vroulike deelnemers tussen die ouderdomme 20-60 jaar is volgens die MetS definisie van die Internasionale Diabetes Federasie (IDF), asook die liggaamsvorme (androïed/genoïed) deur middel van fotoskopiese somatotipering, geklassifiseer. ’n Reeks ondersoeke is gedoen insluitend bloeddruk, antropometrie, bio-elektriese impedansie (BIA), en bloed analises. Bloed analises het vastende glukose, insulien, lipied profiel, insulien-agtige groeifaktor-1 (IGF-1), inflammatoriese merker (C-reaktiewe proteïen (CRP)); en geslagshormoon parameters (estrogeen, vroulike testosteroon, geslags hormoon bindings globulien; en vry-androgeen indeks) ingesluit.

Resultate: Die voorkoms van die MetS was 57.5 %; waaronder abdominale vetsug (73.8 %), verhoogde bloeddruk (BP, 68.8 %), en hoë digtheids lipoproteïen cholesterol (HDL-c) vlakke (68.8 %) die mees prevalente risiko faktore was. Beide die metaboliese status; en liggaamsvorm alleen, is as moontlike voorspellers geïdentifiseer wat die antropometriese, BIA, fisiologiese en biochemiese parameters aanbetref. Die metaboliese status het verder ’n effek op verskeie parameters in die genoïede liggaamsvorm groep getoon, i.e. liggaamsmassa (LM) ($p<0.001$), heup omtrek (HO) ($p<0.01$), liggaamsmassa indeks (LMI) ($p<0.001$), vet massa (VM) (%) ($p<0.01$), vetvrye massa (VVM) (%) ($p<0.01$), middellyf omtrek (MO) ($p<0.001$), HDL-c ($p<0.001$), trigliseriede (TG) ($p<0.05$), sistoliese bloeddruk (SBD) ($p<0.05$) en diastoliese bloeddruk (DBD) ($p<0.01$), terwyl die metaboliese status ’n effek getoon het op LM ($p<0.001$), LMI ($p<0.01$), TG ($p<0.05$), SBD ($p<0.01$) en DBD ($p<0.01$) in die androïede liggaamsvorm groep. Beide die metaboliese status en liggaamsvorm het geen effek op die LVI, totale cholesterol (TC), lae digtheids lipoproteïen cholesterol (LDL-c), vastende insulien, CRP en alle geslagshormoon parameters getoon nie. Betekenisvolle korrelasie analises is verkry onder verskeie antropometriese, BIA en bloed parameters.

Gevolgtrekking: Hierdie studie toon dat die metaboliese status, liggaamsvorm, en/of beide veranderinge in verskeie liggaamssamestelling, fisiologiese en biochemiese parameters in vroue kan voorspel. Geen effekte is waargeneem vir enige parameter wat die MetS met kanker risiko kan verbind nie. Dus, kan geen akkurate afleiding gemaak word oor die patofisiologie hiervan nie. Ons bevindinge oor die LVI noodsaak verdere ondersoek met betrekking tot die MetS, liggaamsvorm en kanker risiko.

Sleutelwoorde: Metaboliese sindroom, liggaamsvorm, kanker

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LIST OF SYMBOLS

Alpha	α
Beta	β
Degrees Celsius	°C
Delta	Δ
Female	♀
Male	♂
Microgram	µg
Microlitre	µl
Nanogram	ng
Percentage	%
Picogram	pg

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LIST OF ABBREVIATIONS

17β-HSD	17β-hydroxyl steroid dehydrogenase
A/G ratio	Android-gynoid ratio
AACE	American Association of Clinical Endocrinology
ABSI	A body shape index
AFM	Android fat mass
AHA	American Heart Association
ANOVA	Analysis of variance
Apn	Adiponectin
BIA	Bio-electrical impedance analysis
BM	Body mass
BMI	Body mass index
BP	Blood pressure
CANSA	Cancer Association of South Africa
CHD	Coronary heart disease
CI	Confidence interval
CRP	C-reactive protein
CT	Computed tomography
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DEXA	Dual-energy X-ray absorptiometry
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
E1	Oestrone
E2	Oestradiol
EGIR	European Group for the Study of Insulin Resistance
ELISA	Enzyme-linked immunosorbent assay
FAI	Free androgen index
FBG	Fasting blood glucose
FFA	Free fatty acids

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FFM	Fat free mass
FFMI	Fat free mass index
FM	Fat mass
FPG	Fasting plasma glucose
GCP	Good clinical practice
GFM	Gynoid fat mass
GH	Growth hormone
GPAQ	Global physical activity questionnaire
HC	Hip circumference
HDL-c	High density lipoprotein-cholesterol
HIF-1α	Hypoxia inducible factor-1α
HPCSA	Health Professions Council of South Africa
HPV	Human papilloma virus
HREC I	Health Research Ethics Committee I
hsCRP	High sensitivity C-reactive protein
IAS	International Atherosclerosis Society
IASO	International Association for the Study of Obesity
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGF-1	Insulin-like growth factor-1
IGF-1R	Insulin-like growth factor-1-receptor
IGF-2	Insulin-like growth factor-2
IGF-BP	Insulin-like growth factor-binding protein
IGT	Impaired glucose tolerance
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IQR	Inter quartile range
IR	Insulin resistance
ISAK	International Society for the Advancement of Kinanthropometry
ISR	Insulin receptor

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JIS	Joint interim statement
LDL-c	Low density lipoprotein cholesterol
M1	Pro-inflammatory macrophage
M2	Anti-inflammatory macrophage
MAPK	Mitogen activated protein kinase
MetS	Metabolic syndrome
MetSA	Metabolic syndrome android group
MetSG	Metabolic syndrome gynoid group
MRI	Magnetic resonance imaging
n	Sample
NA	Not applicable
NCEP-ATPIII	National Cholesterol Education Program-Adult Treatment Panel
NHANES	National health and nutrition examination survey
NHLBI	National Heart, Lung, and Blood Institute
NMetS	Non-metabolic syndrome group
NMetSA	Non-Metabolic syndrome android group
NMetSG	Non-Metabolic syndrome gynoid group
ns	Not significant
OR	Odds ratio
PI3K	Phosphoinositide-3-kinase
PKB	Protein kinase B
RAAS	Renin angiotensin aldosterone system
ROS	Reactive oxygen species
rpm	Revolutions per minute
RR	Relative risk
SAT	Subcutaneous adipose tissue
SBP	Systolic blood pressure
SEM	Standard error of the mean
SF	Sodium fluoride
SHBG	Sex hormone binding globulin
SST	Serum separator tubes
T	Testosterone

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T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TBF %	Total body fat percentage
TBW	Total body water
TC	Total cholesterol
TG	Triglycerides
TGF-β	Transforming growth factor-beta
TMB	3, 3′, 5, 5′-tetramethylbenzidine
TNF-α	Tumour necrosis factor-alpha
USA	United States of America
VAT	Visceral adipose tissue
VEGF	Vascular endothelial growth factor
vs	Versus
WC	Waist circumference
WHF	World Heart Federation
WHO	World Health Organization
WHR	Waist-hip-ratio
ΔA4	Δ4-androstenedione

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

1.1.1 Metabolic syndrome and cancer

Following, and adapting to a westernized lifestyle (unhealthy eating habits, dietary changes (nutrition transition), and decreased physical activity) has increased significantly in both developed and developing countries worldwide (Oggioni *et al*., 2014; Vorster *et al*., 2011). This in turn has contributed to an increase in the incidence of lifestyle diseases such as cardiovascular disease (CVD), diabetes mellitus (DM), and cancer (Tachang *et al*., 2012; WHO, 2014). Annually, lifestyle diseases contribute to 38 million deaths globally, with CVD estimated at 17.5 million deaths per annum, cancer at 8.2 million, and diabetes at 1.5 million deaths (WHO, 2014). Sub-Saharan Africa is currently experiencing an epidemiological transition, characterized by both a high incidence of infectious diseases and increased incidence of lifestyle-related diseases (Statistics South Africa, 2013).

The metabolic syndrome (MetS), regarded as a major risk factor for chronic diseases of lifestyle, shows a global increase in the prevalence of the MetS annually (Kaur, 2014; O'Neill & O'Driscoll, 2015). The MetS, consisting of a cluster of metabolic, physiological and biochemical risk factors, is independently associated with CVD, type 2 diabetes mellitus (T2DM), and has also recently been associated with certain cancers (Beltrán-Sánchez *et al*., 2013). Scientific evidence also links the MetS and its components as important risk factors for the development of various lifestyle cancers (Agnoli *et al*., 2010; Stocks *et al*., 2015). However, the pathophysiology and molecular mechanisms underlying the MetS, and cancer are still poorly understood and remain to be elucidated. Currently, evidence on the pathophysiology of the MetS and cancer development involves several factors which include insulin resistance (IR), inflammation, sex hormones and growth factors, as well as fat distribution. Obesity, specifically abdominal obesity, is implicated as the underlying factor in the development of IR, MetS and also more recently to cancer development (Mendonça *et al.*, 2015; Sinicrope & Dannenberg, 2011).

1.1.2 Anthropometry and body composition

Increased weight, obesity, and fat distribution patterns, i.e. android and gynoid body shape, have been associated with an increased risk for developing metabolic-related diseases and cancer, especially in women (Ronco *et al*., 2008). Various anthropometric measurements have been used to investigate obesity and fat distribution. These typically include body mass index (BMI), waist circumference (WC), waist-to hip-ratio (WHR), and total body fat percentage (TBF %) (Eston & Reilly, 2009; Stewart & Sutton, 2012). However, due to limitations and discrepancies of these anthropometric measurements, a new metric, a body shape index (ABSI), have been proposed to assess the association

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between fat distribution (body shape), and the risk of developing metabolic diseases and certain cancer types (Krakauer & Krakauer, 2012). Therefore, the use of appropriate anthropometric measurements may be helpful in identifying individuals with an increased non-communicable disease risk.

LITERATURE REVIEW

1.2 The metabolic syndrome

1.2.1 History

The MetS is a concept that has been around for more than five decades (Okafor, 2012). Kylin (1923) observed an association between hyperglycaemia, increased arterial blood pressure and gout. In 1965, Avogaro and colleagues provided a similar description of the syndrome (Avogaro *et al*., 1965). Almost 20 years later, Jean Vague described these factors, and its association with visceral obesity, and linked this with metabolic abnormalities found in CVD and T2DM (Vague *et al*., 1989; Vague, 1996). During the late 1980's, Reaven gave the famous Banting lecture, and although it was proposed that IR was the underlying factor of these metabolic abnormalities (referred to as Syndrome X), abdominal obesity was not included in this concept (Reaven, 1988). Currently, this concept is known as the MetS, a clinical term which remains the most widely used (Kaur, 2014; Okafor, 2012; Thaman & Arora, 2013).

1.2.2 The metabolic syndrome and components

The MetS is clinically described as a cluster of metabolic, physiological and biochemical risk factors that functions in an interconnected manner. This cluster of risk factors has been shown to increase the risk of developing cardiovascular-related dysfunction, T2DM, as well as all-cause mortality, both as a unit (Kassi *et al*., 2011; Kaur, 2014), as well as independently (Beltrán-Sánchez *et al*., 2013). The MetS risk factors are typically described in terms of body dimensions, blood lipid profiles, blood pressure, blood glucose homeostasis, and can also include inflammatory and pro-thrombotic profiles depending on what definition of the syndrome is used (Kaur, 2014; Thaman & Arora, 2013). The main components of the MetS (Figure 1.1) includes, obesity (specifically abdominal obesity), dyslipidaemia (increased triglycerides (TG), low density lipoprotein cholesterol (LDL-c), Apo-lipoprotein and decreased high density lipoprotein-cholesterol (HDL-c)), increased arterial blood pressure (systolic and diastolic), IR and impaired blood glucose homeostasis (Alberti *et al*., 2009; Després *et al*., 2008; Kassi *et al*., 2011; Kaur, 2014; Okafor, 2012).

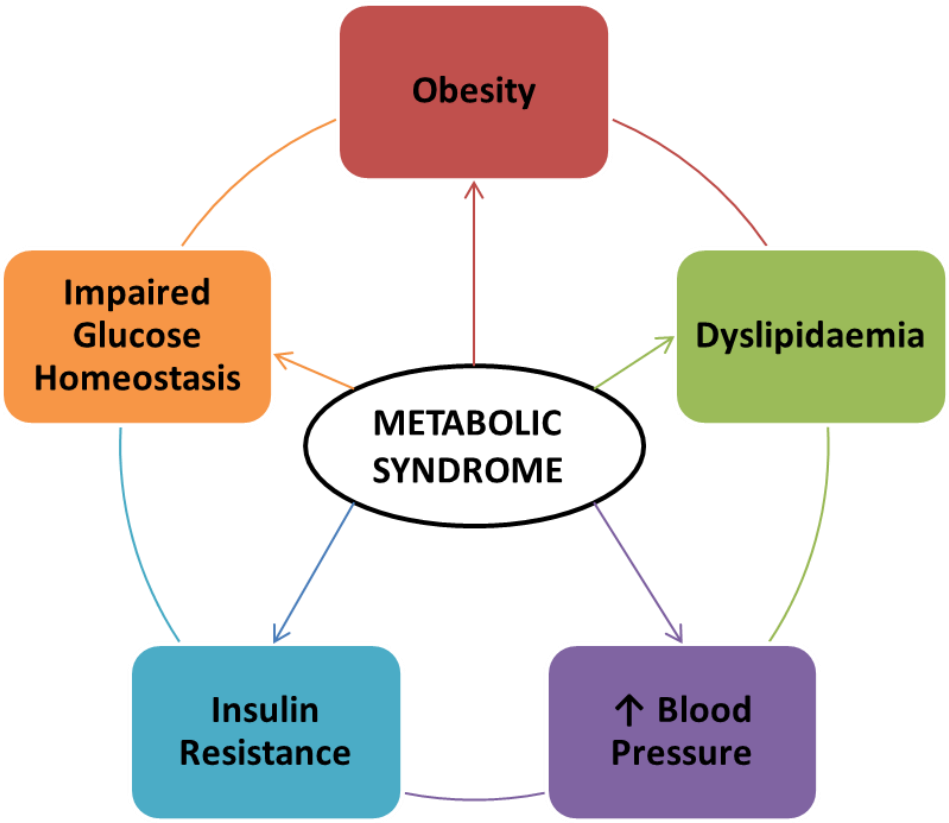

Figure 1.1: Main components of the MetS.
(Adapted from Després *et al*., 2008; Okafor, 2012)

Other physiological factors such as pro-inflammatory markers, pro-thrombotic markers, sleep apnea, and non-alcoholic fatty liver disease, have also been considered, adding to the complexity of the MetS (Alberti *et al*., 2009, Kassi *et al*., 2011; Kaur, 2014; Okafor, 2012). Abdominal obesity and IR have been implicated as the primary factors in the development of the MetS. However, due to the complexity of the MetS, there is still no unifying definition which clearly defines the MetS and its diagnostic criteria (Kassi *et al.*, 2011), since different definitions of the MetS exist.

1.2.3 Definitions

Table 1.1 summarizes the different definitions of the MetS available to date. During 1998, the World Health Organization (WHO) defined the MetS by including IR, impaired glucose tolerance, or the presence of T2DM in their definition (Alberti & Zimmet, 1998). This, in combination with two or more of the other defined criteria, finalized their definition of the MetS (Alberti & Zimmet, 1998). A year later, the European Group for the Study of Insulin Resistance (EGIR) discarded the use of micro-albumin as criteria for the MetS, and instead added hyperinsulinaemia (Balkau & Charles, 1999). The EGIR also discarded the use of the BMI as a measurement of obesity, and instead added the WC.

Table 1.1 Different metabolic status definitions and defined criteria

Criteria		WHO	EGIR	NCEP-ATPIII	AACE	AHA/NHLBI	IDF	JIS
Definition		T2DM/ IFG/ IGT/IR and or ≥ 2 of the other criteria	T2DM/ IFG/ IGT/IR and or ≥ 2 of the other criteria	≥ 3 of 5 criteria, does not consider IR	IGT/IFG plus any 2 of the other criteria	≥ 3 of 5 criteria, does not consider IR	Central obesity plus ≥ 2 of the other criteria	≥ 3 of 5 criteria, does not consider IR
Diabetes		Yes	No	Yes	No	Yes	Yes	Yes
Glucose measurements		IGT, IFG or T2DM	FPG: ≥ 6.1 mmol/L	FPG: ≥ 6.1 mmol/L	FPG: ≥ 6.1 mmol/L	FPG: ≥ 5.6 mmol/L or on diabetes treatment	FPG: ≥ 5.6 mmol/L or T2DM diagnosis	FPG: ≥ 5.6 mmol/L or on diabetes treatment
Insulin resistance		Glucose uptake below lowest quartile	IR or Insulin levels > 75th percentile	NA	NA	NA	NA	NA
Anthropometry		WHR: > 0.90 men & > 0.85 women BMI: ≥ 30 kg/m^2 men & women	WC: ≥ 94cm men & ≥ 80 cm women	WC: >102 cm men & > 88 cm women	BMI: ≥ 25 kg/m^2 men & women	WC: ≥ 102 cm men & ≥ 88 cm women	Central obesity:↑ WC (ethnic specificity)	WC: population and country specific
Lipid Profile	**TG**	≥ 1.7 mmol/L and or HDL-c	≥ 1.7 mmol/L and or HDL-c	≥ 1.7 mmol/L and or HDL-c	≥ 1.7 mmol/L and or HDL-c	≥ 1.7 mmol/L and or HDL-c	≥ 1.7 mmol/L or on TG treatment and or HDL-c	≥ 1.7 mmol/L and or HDL-c
	HDL-c	< 0.90 mmol/L men & < 1.00 mmol/L women	< 1.00 mmol/L men & women	< 1.03 mmol/L men & < 1.30 mmol/L women	< 1.03 mmol/L men & < 1.30 mmol/L women	< 1.03 mmol/L men & < 1.30 mmol/L women	< 1.03 mmol/L men & <1.30mmol/L women or on HDL-c treatment	< 1.03 mmol/L men & < 1.30 mmol/L women or on HDL-c treatment
Arterial Blood Pressure		≥ SBP 140, DBP ≥ 90 mmHg	≥ SBP 140, DBP ≥ 90 mmHg or hypertension treatment	≥ SBP 130, DBP ≥ 85 mmHg	≥ SBP 130, DBP ≥ 85 mmHg	≥ SBP 130, DBP ≥ 85 mmHg	≥ SBP 130, DBP ≥ 85 mmHg or on hypertension treatment	≥ SBP 130, DBP ≥ 85 mmHg or hypertension treatment
Other		Micro albuminuria: ≥ 20 µg/min or albumin creatine ≥ 20 µg/min or	NA	NA	Other feature of IR based on clinical judgment	NA	NA	NA

(Adapted from: Alberti & Zimmet, 1998; Alberti *et al*., 2009; Balkau & Charles, 1999; Einhorn *et al.*, 2003; Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001; Ford *et al.*, 2010; Grundy *et al.*, 2004; IDF, 2006)

During 2001, the National Cholesterol Education Program-Adult Treatment Panel (NCEP-ATPIII) proposed new cut-off values for WC, blood lipid profiles, arterial blood pressure, and fasting blood glucose (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001). In contrast to the WHO and EGIR, the NCEP-ATP III excluded IR as diagnostic criteria due to the cost and difficulty of implementing gold standard tests for IR in a clinical setting.

The American Association of Clinical Endocrinology (AACE) published a set of criteria (Einhorn *et al.*, 2003), which was followed by a publication from the American Heart Association (AHA) and the National Heart, Lung, and Blood Institute (NHLBI) in 2004 (Grundy *et al.*, 2004). In 2005, the International Diabetes Federation (IDF) published an updated set of criteria, in an effort to serve as a universal diagnostic tool in both a clinical and research setting (IDF, 2006). In this definition, abdominal obesity was included as the primary diagnostic criteria since body weight and WC differences exist between populations, different ethnic groups and individuals with different nationalities (IDF, 2006) (see Table 1.2). These differences therefore need to be taken into consideration when determining the presence of the MetS.

Table 1.2: IDF: Ethnic-specific values for waist circumference

Ethnic Background	Men	Women
Europeans	≥ 94 cm	≥ 80 cm
South-Asians	≥ 90 cm	≥ 80 cm
Chinese	≥ 90 cm	≥ 80 cm
Japanese	≥ 90 cm	≥ 80 cm
South and Central Americans	Use South- Asian values	
	≥ 90 cm	≥ 80 cm
Sub-Saharan Africans	Use European values	
	≥ 94 cm	≥ 80 cm
Eastern Mediterranean and middle east populations	Use European values	
	≥ 94 cm	≥ 80 cm

(Adapted from: Alberti *et al*., 2009; IDF, 2006)

In 2009, a joint interim statement (JIS) was published by the IDF, AHA/NHBLI, World Heart Federation (WHF), International Atherosclerosis Society (IAS), and the International Association for the Study of Obesity (IASO) (Alberti *et al*., 2009). This definition still comprised of the main components of the MetS, but does not declare that any of the components are required for diagnostic properties of the MetS (Alberti *et al*., 2009).

Therefore, although the different definitions agree on the core components of the MetS (obesity, IR, dyslipidaemia, hypertension and high blood glucose), there are several discrepancies between these definitions, which complicates the comparability of different studies, as well as to define cut-off values for different populations (Kaur, 2014). The most widely used definitions are those formulated by the IDF and NCEP-ATPIII, which include central obesity, while the AACE, WHO and EGIR's definition focused more on IR (Huang, 2009).

Definitions using IR as a criterion (WHO & EGIR) are criticized by the fact that the gold standard method (hyperinsulinaemia-eugycaemic clamp) to test for IR cannot be implemented as a routine test,

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due to the fact that it is time consuming, costly, and labor intensive (Abdul-Ghani *et al*., 2007; Okafor, 2012). Conversely, definitions that do not include IR (NCEP-ATPIII, ACCE, IDF and JIS) can be easily implemented in a routine clinical setting (Huang, 2009; Okafor, 2012). More specifically, a study showed that the use of the NCEP-ATPIII, IDF and ACCE definitions are more useful that those definitions which require IR, since more individuals are clinically identified with the MetS, IR and at risk for CVD (Can & Bersot, 2007). However, the use of the WHO and NCEP-ATPIII is problematic in terms of their applicability to different countries and ethnic groups especially in determining obesity cut-off values (Kassi *et al*., 2011).

Although different definitions have been proposed which highlights some discrepancies, the MetS is still recognized as a global epidemiological health problem which is fueled by an increase in sedentary lifestyles and unhealthy diets (Tachang *et al*., 2012).

1.2.4 Epidemiology

The epidemiology of the MetS is topical due to a lack of consensus in defining the syndrome, the components of the syndrome itself, and the specific cut-off values for different countries and cultures (Kaur, 2014). In order to determine the prevalence of the MetS, the definition used, sample size, the region of interest, the population being studied (i.e. gender, age, and ethnicity), as well as the type of environment (i.e. urban or rural) should all be taken into consideration (Prasad *et al.*, 2012). Other factors have also been proposed to play a role in the epidemiology of the MetS. This includes socio-economic status, sedentary lifestyles, level of physical activity and genetic factors (Popkin *et al.*, 2012)

Regardless of the MetS definition and cut-off values used, as well as the impact of various factors associated with the MetS, the prevalence of the MetS is significantly high and is on the rise in most populations around the world (Beltrán-Sánchez *et al.*, 2013; Bhanushali *et al.*, 2013; Cameron *et al.*, 2007; Emem-chioma & State, 2008; Erasmus *et al.*, 2012; Hu *et al.*, 2004; Lim *et al.*, 2011; Motala *et al.*, 2011; Prasad *et al.*, 2012; Tran *et al.*, 2011).

1.2.4.1 Global prevalence of the MetS

Globally, the prevalence of the MetS is estimated to range between less than ten percent to 62 % (see Table 1.3). This increase in the MetS prevalence has also been exacerbated by the increasing incidence of obesity (WHO, 2014). Epidemiological studies in the United States of America (USA) found that the prevalence of the MetS ranged between 20-40 % as a function of gender, and also depending on the definition used (Beltrán-Sánchez *et al.*, 2013; Bhanushali *et al*., 2013). Bhanushali *et al*. (2013) found that the MetS was more prevalent in women compared to men (39.4 % *vs* 26.8 % respectively) using the NCEP-ATPIII definition. This higher prevalence amongst the women can be explained by

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the fact that certain risk factors are more prevalent in women than men, such as abdominal obesity and HDL-c levels (Bhanushali *et al*., 2013).

Beltrán-Sánchez *et al*. (2013) found that a fifth of the USA population remained at a high risk for cardio-metabolic diseases. Also, even though the MetS prevalence decreased significantly over time from 25.5 % between 1999-2000, to 22.9 % between 2009-2010 (p=0.024), a fifth of this population still presented with the MetS, since they still exhibited at least three to five of the MetS risk factors. Furthermore, this study found gender and racial differences, i.e. white men had a higher prevalence of abdominal obesity *vs* their other racial counterparts and black women had a higher prevalence of elevated blood pressure (Beltrán-Sánchez *et al*., 2013).

A European study which included data from 11 European study cohorts (n=11507 individual data sets; n=6151 men and n=5351 women) reported an overall prevalence of between 6.3-29.9 % in women *vs* 7.7-35.5 % in men depending on the definition used (Hu *et al*., 2004). However, when the authors used a modified version (hyperinsulinaemia was added, and micro-albumin was excluded) of the WHO definition for the MetS, they found that the prevalence increased in both men (15 %) and women (14.2 %) (Hu *et al*., 2004). This study further showed that the prevalence of the MetS changed with the addition of more risk factors to the definition. Additionally, Hu *et al.* (2004) also found that the prevalence of the MetS (as defined by the WHO) was associated with a 1.4 fold increase in the risk of all-cause mortality in both genders, as well as a 2.3 fold increase in the risk for CVD mortality in men, and 2.8 fold in women. A Korean study showed increased MetS prevalence from 24.9 % in 1998, to 31.3 % in 2007 (Lim *et al*., 2011).

Similar results were observed in a study by Prasad *et al*. (2012), where 33.5 % of an Indian population presented with the MetS. The MetS in this population also differed in terms of gender, with a higher prevalence seen in women *vs* men (42.3 % *vs* 24.9 % respectively). In addition, those individuals classified as having the MetS, lead a sedentary lifestyle, had low fruit intake, were obese, presented with abdominal obesity and displayed increased TG and LDL-c levels (Prasad *et al*., 2012).

An Australian study by Cameron *et al.* (2007) included the WHO, EGIR, NCEP-ATPIII and IDF definitions to determine the prevalence of the MetS (Cameron *et al*., 2007). They found that the prevalence of the MetS ranged between 13.4-30.7 % depending on the definition used, i.e. WHO (21.7 %), EGIR (13.4 %), NCEP-ATPIII (22.1 %), and IDF (30.7 %) respectively. They also showed that the prevalence of the MetS increased with an increase in age for all the definitions used, and that the prevalence was greater in men than women irrespective of which definition was used (Cameron *et al*., 2007).

Irrespective of the definition used, and what region of the world the MetS is investigated in, the prevalence of MetS is high with distinct gender and ethnic differences. This is worrying, especially since the MetS may be well studied in some regions of the world, which is not the case for Africa.

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This therefore indicates that more studies investigating the MetS, especially in the South African continent, are needed in order to fully understand the potential burden of the MetS and its associated health risks.

1.2.4.2 African prevalence of the MetS

Limited epidemiological data exists on the prevalence of the MetS on the African continent. This could largely be due to the lack of an African-applicable MetS definition (Okafor, 2012) and the fact that WC cut-off values, specific for African people have not yet been established (Motala *et al*., 2011). Most South African studies to date have been based on definitions and cut-off values derived from studies conducted in North-America, Europe and Asia (Miranda *et al*., 2005; Thaman & Arora, 2013; Tran *et al.*, 2011). This in turn has implications of establishing a MetS definition that can be used in an African setting (Okafor, 2012). However, recent South African studies with a good representable sample of the MetS, advocated for South African-specific WC cut-off values (Motala *et al.*, 2011; Peer *et al*., 2015[B]).

The prevalence of the MetS in Africa is estimated to range between less than five percent and 18.5 % (see Table 1.3). Emem-chioma & State (2008) reported that approximately six percent of the participants presented with the MetS using the NCEP-ATPIII criteria. Furthermore, the men displayed a higher MetS prevalence when compared to women. In addition, abdominal obesity, hypertension and low HDL-c were all significant risk factors associated with the MetS in this population (Emem-chioma & State, 2008). Awosan and colleagues (2013) reported a third of the participants had central obesity or an increased WC. Additionally, although an overall 37.8 % had reduced HDL-c, 32.8 % had increased TG levels, and 31.9 % had high blood pressure, these percentages were higher in men. In this specific study, the overall prevalence of the MetS ranged between 17.8 and 18.5 % depending on the definition used. Furthermore, this study also highlighted the IDF definition as a much more sensitive definition of identifying the MetS in the included study participants (Awosan *et al.*, 2013).

An Ethiopian study found the overall prevalence of the MetS to be 12.5 % using the NCEP-ATPIII, and 17.9 % using the IDF definition (Tran *et al.*, 2011). A significant increase in BMI and arterial blood pressure were more prominent in women than men, which suggests that women are at greater risk for the development of the MetS (16.2 *vs* 10.0 %, NCEP-ATPIII & 24.0 *vs* 14.0 %, IDF) (Tran *et al.*, 2011).

Table 1.3: Prevalence of the MetS: Global to South Africa

Author	Country	Total	Metabolic syndrome definition						MetS prevalence outcomes
			WHO	*EGIR*	*NCEP-ATPIII*	*IDF*	*JIS*	*Other*	
Bhanushali *et al.*, 2013	USA	n= 1326			√				More prevalent in women (39.43 %) than men (26.77 %).
Beltrán-Sánchez *et al.*, 2013	USA	n= 10 823					√		Prevalence between 20-30 %.
Hu *et al.*, 2004	Europe	n= 11 507	**Modified**					√	Prevalence between 7.7-35.5 % in men and 6.3-29.9 % in women, depending on definition used.
Prasad *et al.*, 2012	India	n= 1178					√		Prevalence was high overall (33.5 %), with a higher prevalence in women (42.3 %) *vs* men (24.9 %).
Lim *et al.*, 2011	Korea	n= 6907			√				Prevalence between 24.9-31.3 % from 1998-2007, thus a 6.4% increase in MetS.
Cameron *et al.*, 2007	Australia	n= 1326	√	√	√	√			Prevalence between 13.4-30.7 % depending on definition.
Emen-chioma & State, 2008	Nigeria	n= 300			√				Prevalence of 6.3 %.
Awosan *et al.*, 2013	Nigeria	n= 270			√	√			Prevalence between 17.8-18.5 % depending on definition.
Tran *et al.*, 2011	Ethiopia	n= 1935			√	√			Prevalence between 12.5-17.9 % depending on definition
Tachang *et al.*, 2012	Cameroon	n= 147	√		√	√			Prevalence between < 5-7.5 % depending on definition.
Motala *et al.*, 2011	South Africa (KwaZulu-Natal)	n= 947			√	√	√		Crude prevalence was 26.5% and age-adjusted prevalence between 15-22.1 % depending on definition.
Erasmus *et al.*, 2012	South Africa (Western-Cape)	n= 588			√	√	√		Prevalence between 55.4-62.0 % depending on definition.
Peer *et al.*, 2015[A]	South Africa (Western-Cape)	n= 1099					√		Age-adjusted prevalence in women (44.9%) higher than men (17.3 %).

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From all the studies cited in this section, the most prominent finding was that even though the African-based studies had smaller sample sizes in comparison to the international studies, the prevalence of the MetS was still considered relatively high. This is an important fact to consider, especially since the definitions and cut-off values used were derived from studies conducted in North-America, Europe and Asia. In addition, the prevalence of individual components of the MetS was also high, suggesting that Africans may be at greater risk of developing the MetS and/or other metabolic related diseases in the future.

1.2.4.3 South African prevalence of the MetS

South Africa underwent several political changes, and with this came rapid and significant health transition. These changes were accompanied by lifestyle changes (nutrition transition, eating behaviour, changes in physical activity), all of which have been shown to significantly impact lifestyle diseases such as obesity and hypertension, and there for the MetS (Bourne *et al*., 2002; Erasmus *et al.*, 2012; Steyn *et al*., 2012; van Zyl *et al.*, 2012; Vorster *et al*., 2005; Vorster *et al*., 2011).

A study conducted in the Free-state province aimed to determine the risk profile of chronic lifestyle diseases and the MetS in an urban and rural setting (van Zyl *et al*., 2012). Here, a high prevalence of hypertension was reported in the rural community participants', with fewer participants displaying a BMI higher than 25 kg/m^2 in the urban community (53.0% *vs* 54.2 %) Obesity was also found to be a major problem in the women, particularly those from the rural community (57.6 % had a WC of $\geq$ 88cm and 43.1 % had a BMI of $\geq$ 30 kg/m^2) (van Zyl *et al*., 2012). However, irrespective of whether the participants were from a rural/urban setting, a high prevalence of the individual MetS components were observed, especially in women.

A cross-sectional study from the Ubombo district, KwaZulu Natal, determined the prevalence of the MetS in an apparently healthy rural African community (Motala *et al*., 2011). The overall prevalence of the MetS, depending on the definition used, differed, i.e. JIS (26.5 %), IDF (23.3%) and NCEP-ATPIII (18.5 %), with an overall higher prevalence in women *vs* men. After adjusting for age, the prevalence was lower than that initially observed (JIS (22.1 %), IDF (19.2 %) and NCEP-ATPIII (15.0 %)). The JIS definition was also considered a more sensitive tool compared to the IDF and NCEP-ATPIII definitions respectively. Upon closer investigation of the individual MetS risk factors, approximately 65.2 % of women displayed low HDL-c levels, whereas 47.1% of men had elevated systolic blood pressure. The high prevalence of the MetS in the women of this study was proposed to be related to a higher prevalence of obesity (increased WC), a lack of physical activity as well as dietary-related factors (Motala *et al*., 2011). This study was also the first to suggest optimal WC cut-off values for Africans (86 cm for men and 92 cm for women) (Motala *et al*., 2011).

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A study completed in the Western-Cape reported that BMI, WC, arterial blood pressure, total cholesterol (TC) and HDL-c were significantly higher in women, while blood pressure and LDL-c were significantly higher in men (Erasmus *et al.*, 2012). A similar pattern of the MetS prevalence was reported (depending on definition) to that of Motala *et al.* (2011). However, the overall prevalence of the MetS was significantly higher in this population; 62 % (JIS), 60.6 % (IDF) and 55.4% (NCEP-ATPIII) respectively (Erasmus *et al.*, 2012).

More recently, a study by Peer *et al*. (2015[A]) found that the age-adjusted prevalence of the MetS in an urban black population in Cape Town was 44.9 % in women, and 17.3 % in men according to the JIS definition (Peer *et al.*, 2015[A]). In addition, the most prevalent components of the MetS among women were central obesity (86 %) and low HDL-c (75 %), while in men, high blood pressure (51.4 %) was the main contributor overall. The prevalence found in this study was also much higher than that reported by Motala *et al*. (2011), which also made use of the JIS definition. They also showed that the prevalence of MetS increased with age.

Another study by Peer *et al*. (2015[B]) reported optimal WC cut-off values based on a black population from Cape Town (83.9 cm men and 94 cm women) and verified the findings of Montala *et al.* (2011). However, these cut-off values differed from what was initially recommended for Africans (Alberti *et al*., 2009; IDF, 2006). The studies by Peer *et al.* (2015[B]) and Motala *et al.* (2011) therefore suggested that current WC cut-off values for Africans may require change or adaptations depending on the population used and the specific setting. The higher WC cut-off values in women highlights the importance of ethnic differences in body composition. Since, black women have less visceral adipose tissue (VAT) compared to white women, for a given WC (Sumner *et al*., 2011). In addition, South African studies have also shown that women have higher WC *vs* men (Erasmus *et al.*, 2012; van Zyl *et al*., 2012). However, even though this may explain the higher WC cut-off values in women *vs* men; further investigation is required to support these findings.

The studies considered in this section clearly emphasize the need for South Africans to recognize the burden of chronic lifestyle diseases. Evidence shows that the prevalence of the MetS is high in all regions of the world, including South Africa. Clear differences are seen with respect to different ages, ethnicity, gender, geographical area, as well as the MetS definition used. Furthermore, the prevalence of the MetS and its individual components are still high, especially in women. Previous evidence also shows that the MetS and its components increase the risk to not only develop CVD and T2DM, but also increases mortality (Kassi *et al.*, 2011; Kaur, 2014).

Experimental and cross-sectional studies now also link the MetS and its individual components to the development and progression of various lifestyle cancer types (Donohoe *et al*., 2011; O'Neill & O'Driscoll, 2015; Zhu *et al*., 2010).

1.3 Pathophysiology of the metabolic syndrome and the link to cancer

1.3.1 Introduction

Emerging evidence links the MetS and its components as important risk factors, in addition to traditional lifestyle risk factors, for the development of various cancer types (O'Neill & O'Driscoll, 2015) (Figure 1.2). Traditional lifestyle-associated cancer risk factors include dietary factors, addictive substances, and low physical activity levels (WHO, 2009; WHO, 2014). Dietary components, for example increased fat, salt, refined sugar, and processed and red meat intake have all been strongly associated with an increased risk of developing cancer at different anatomical sites (Lee & Derakhshan, 2013). This is in contrast to a diet rich in fresh produce, fruit, vegetables, fibre and micronutrients, such as vitamin C and calcium, which are thought to be cancer protective (Esposito *et al*., 2014).

In addition to changes in dietary patterns, the prevalence of sedentary behavior is increasing worldwide (Dias *et al*., 2014). Increased physical activity levels have been shown to reduce cancer risk; however, there is still a lack of evidence to support this association (Brown *et al*., 2012). Some evidence on addictive substance use exists. It has been shown that heavy, or regular alcohol consumption and tobacco use, are independent risk factors for cancer development in various physiological systems, including the respiratory, digestive and urinary systems (O'Neill & O'Driscoll, 2015; Touvier *et al*., 2014). However, the combination of regular or heavy alcohol consumption with tobacco use, contributes to an even greater cancer risk (Touvier *et al*., 2014).

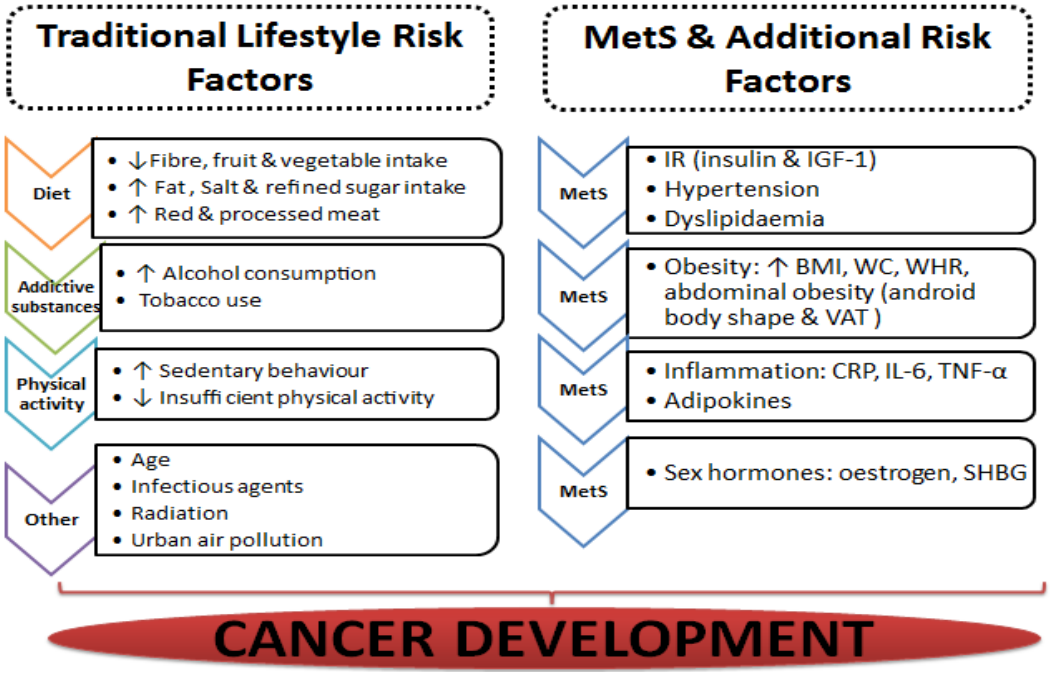

Figure 1.2: Traditional and other risk factors associated with the pathophysiology of cancer development.
(Adapted from: Baan *et al*., 2007; de Haas *et al*., 2010; Weiderpass, 2010)

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Other traditional risk factors include, but are not limited to, age, air pollution, radiation exposure, and viral vectors (WHO, 2009), such as hepatitis B and C (liver cancer) and human papilloma virus (HPV) (cervical cancer) (Esposito *et al*., 2014).

The MetS, and its components (hypertension, dyslipidaemia, IR, and abdominal obesity), are thought to be primary factors in the pathophysiology and risk for developing both CVD and lifestyle associated cancers (Arcidiacono *et al*., 2012; Bjørge *et al*., 2011; Donohoe *et al*., 2011).

1.3.2 Evidence of the MetS and its link to specific (lifestyle) cancers

The MetS as a single entity, and its components, has recently been implicated in cancer risk and development (Stocks *et al*., 2015; Ulmer *et al*., 2012). Numerous studies describes a link between the MetS and its components to an increased risk for various cancers such as colorectal, endometrial, ovarian, breast (post-menopausal), cervical and thyroid cancer (Agnoli *et al*., 2010; Mendonça *et al.*, 2015; Sinicrope & Dannenberg, 2010; Stocks *et al*., 2015). In addition, studies also indicated an increase in cancer incidence and mortality rates amongst MetS patients (Bjørge *et al*., 2011; Esposito *et al*., 2012). Table 1.4 provides scientific evidence linking the MetS to various types of cancer. Here, it is clearly indicated which MetS components is mostly associated with increased risk of cancer development.

Bjørge *et al*. (2011) investigated the association between the MetS, and ovarian cancer, and found that increased levels of cholesterol (relative risk (RR) 1.52 (95 % confidence interval (CI) 1.01-2.29)) and systolic blood pressure (SBP) (RR 1.79 (95 % CI 1.12-2.86)) were positively associated with an increased risk for developing mucinous and endometrioid tumour sub-types, respectively. More interestingly, women younger than 50 years showed an increased risk of ovarian cancer mortality that was associated with the MetS (RR 1.52 (95 % CI 1.00- 2.30)), while an increased BMI conferred with an increased risk for ovarian cancer mortality in women older than 50 years (RR 1.71 (95 % CI 1.01-1.37)) (Bjørge *et al*., 2011). Circulating sex hormones could partly explain the relationship with BMI, since BMI is associated with increased production of peripheral tissue oestrogen (post-menopausal) (Weinberg *et al*., 2006).

In a study by Kim *et al*. (2007), the prevalence of the MetS was reported to be higher in colorectal adenoma cases (17 %) *vs* age-matched controls (11 %). The risk for colorectal adenoma was also significantly increased in participants with the MetS (odds ratio (OR) 1.51 (1.18-1.93); p=0.001)). In addition, abdominal obesity, as measured by WC, was found to be the only independent MetS risk factor for colorectal adenoma (OR 1.39 (1.15-1.68); p=0.001) (Kim *et al*., 2007). This is a definite possibility since WC is a surrogate measure of VAT deposition, and an increase in VAT is associated

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with IR, as well as increased levels of insulin-like growth factor-1 (IGF-1) (Donohoe *et al*., 2011). Both IR and IGF-1 are major determinants of proliferation and apoptosis, and could possibly play a key role in carcinogenesis by increasing proliferation and decreasing apoptosis (Donohoe *et al*., 2011).

Zhang *et al*. (2010[B]) found that women who were classified as being overweight (RR 6.150 (3.976-9.513); $p<0.001$), obese (RR 1.511 (1.262-18.809); $p<0.001$), and having diabetes (OR 2.207 (1.229-3.964); $p=0.0007$) had an increased risk for endometrial cancer when, compared to women with a normal weight. This was found to be true for both endometrial cancer cases and healthy age-matched controls. In addition, TG, TC and LDL-c were positively correlated with endometrial cancer, while HDL-c showed a negative correlation (Zhang *et al*., 2010[B]). An association between IR and an increased risk for endometrial cancer have also been found (Friedenreich *et al.*, 2012).

Ulmer *et al*. (2012) showed that higher BMI, TG and blood pressure were positively associated with an increased risk for cervical cancer. Here, authors concluded that increased adipokine and inflammatory marker levels related to a high BMI and HPV infection may therefore be regarded as possible mechanisms for MetS-associated cervical cancer risk (Ulmer *et al*., 2012).

One of the largest epidemiological studies to date, which included seven European cohorts, investigated the link between the MetS and the risk of various cancer types in both genders (Stocks *et al*., 2015). This cohort showed that BMI, blood pressure, blood glucose, TG and TC were positively associated with an increased risk of cancer incidence in both men and women. The MetS was also positively associated with an increased risk for overall cancer incidence and mortality in several cancers. Men displayed a stronger association for renal and liver cancer, compared to endometrial and pancreatic cancer in women. A positive association was also found for cancers involving the oral cavity in both genders. In addition, since some of the individual components of the MetS were also associated with cancer mortality, evidence suggests that the metabolic factors might be involved in tumour development and progression (Stocks *et al*., 2015)

Table 1.4: A summary of epidemiological studies linking the MetS to specific types of cancer

Author	Study Design	Population	Cancer type	MetS prevalence and/or MetS components	Major findings
Agnoli *et al.*, 2010	Case-control	n=792 Post-menopausal women	Breast (Post-menopausal)	▪ High TG levels and reduced HDL-c associated with an increased risk for breast cancer.	▪ Presence of the MetS associates with increased breast cancer risk.
Alokail *et al.*, 2013	Cross-sectional (Case-control)	n=109 women (53 controls & 56 cases)	Breast (post-menopausal)	▪ Higher SBP, blood glucose, TG and reduced HDL-c levels found in breast cancer cases.	▪ Association between increased TG levels and risk of breast cancer.
Bjørge *et al.*, 2011	Prospective cohort	n=287 320 women	Ovarian	▪ Increased levels of cholesterol and blood pressure conferred with an increased risk of tumour development. ▪ Increased BMI conferred with an increased ovarian cancer mortality risk.	▪ 644 cases of epithelial ovarian cancer cases identified. ▪ 388 ovarian cancer deaths. ▪ Increased levels of cholesterol and blood pressure may play a role in cancer development.
Friedenreich *et al.*, 2012	Case-control	n=1476 women	Endometrial	▪ IR associated with an increased risk for endometrial cancer.	▪ Overall endometrial cancer cases had higher measures of IR *vs* age matched controls. ▪ Age-adjusted analysis showed that increased levels of insulin, and IR were associated with an increased endometrial cancer risk.
Healy *et al.*, 2010	Prospective cohort	n=105 women	Breast (post-menopausal)	▪ High prevalence of the MetS and central obesity in women with post-menopausal breast cancer.	▪ Obesity and the MetS were associated with a larger tumour size, clinical and pathological breast cancer stage.
Kim *et al.*, 2007	Cross-sectional	n=2531 men & women	Colorectal	▪ Higher prevalence of the MetS in colorectal adenoma case *vs* control group. ▪ High WC associated with an increased risk for colorectal adenoma.	▪ Overall association between MetS and colorectal adenoma cancer risk.
Stocks *et al.*, 2015	Pooled analysis	n=564 596 men and women	Several cancer types	▪ BMI, blood pressure, blood glucose, TG and TC associated with a risk of cancer incidence. ▪ Blood pressure, blood glucose, and TG levels associated with cancer mortality.	▪ MetS positively associated with a risk for overall cancer incidence and mortality in several cancer types.
Ulmer *et al.*, 2012	Prospective cohort	n=288 834 women	Cervical	▪ High BMI, blood pressure and TG levels associated with an increased risk for cervical cancer.	▪ 425 cases of cervical cancer ▪ Overall association between the MetS and cervical cancer risk.
Zhang *et al.*, 2010[B]	Case-control	n=2663 women	Endometrial	▪ Overweight, obesity, diabetes, hyperglycaemia, glucose intolerance and dyslipidaemia are associated with an increased risk for endometrial cancer.	▪ Metabolic abnormalities were associated with an increased endometrial cancer risk in the population overall.

An increase in breast cancer incidence has been observed in overweight and obese patients (Healy *et al*., 2010), as well as patients with T2DM (Onitilo *et al*., 2014), and the MetS (Agnoli *et al*., 2010; Healy *et al*., 2010). Agnoli *et al*. (2010) found that the presence of the MetS in post-menopausal women was directly associated with breast cancer risk (RR 1.58 (1.07-2.33)). This risk increased with the presence of three or more components of the MetS, even though some of the individual components of the MetS (TG and HDL-c) were also associated with an increased risk for breast cancer development (Agnoli *et al*., 2010; Alokail *et al*., 2013). Healy *et al*. (2010) on the other hand found that the overall prevalence of the MetS was approximately 40 %, and was more common in obese (61 %) *vs* non-obese patients (21 %). In addition, obesity was positively associated with a larger tumour size (> 2 cm; p=0.021), indicative of a later clinical and pathological breast cancer stage (Healy *et al*., 2010). This increased risk may be partly due to a change in sex hormone levels during menopause (decrease in oestrogen and an increase in androgens), which in turn, is associated with a change in fat distribution patterns, resulting in a more male-like body shape (android body shape and increased VAT). Other studies have also found an association between android body shape and an increase prevalence of the MetS, as well as all the components of the MetS (Noroozi *et al*., 2010; Pradhan, 2014; Sardinha *et al*, 2012).

The role of the MetS as a single entity and/or its components in cancer development still needs to be elucidated, as no single component explains the full risk associated with cancer. A complex pathophysiology between cancer and the MetS and its components is proposed, which may include multiple pathways, as well as biochemical, physiological and genetic factors (Sinicrope & Dannenberg, 2010).

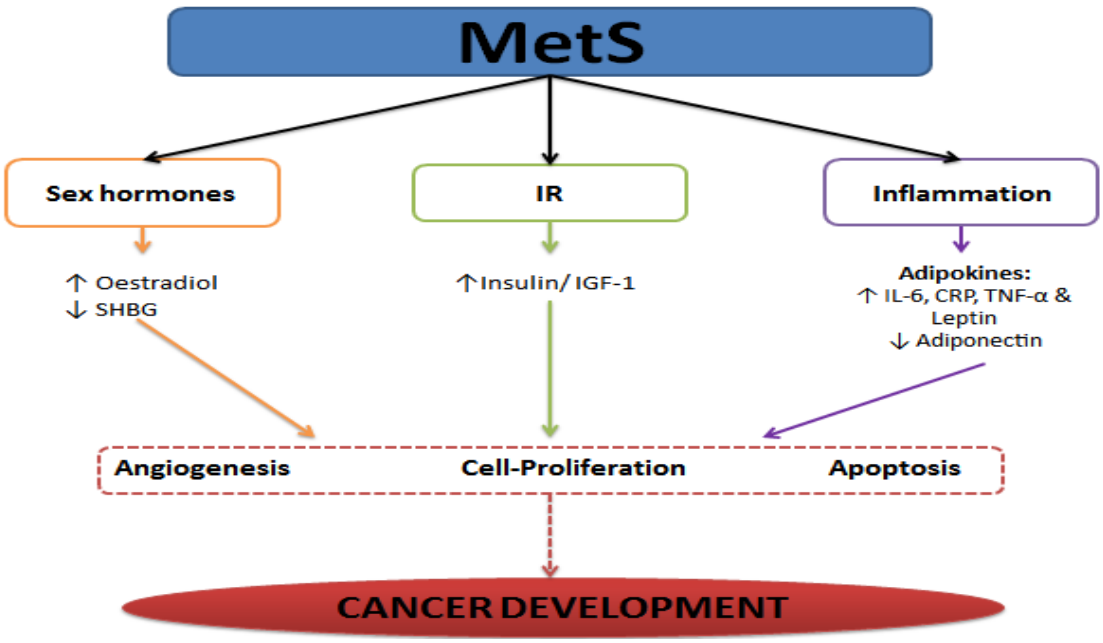

Figure 1.3: A diagram illustrating the proposed pathophysiology of MetS, and the link to cancer.
(Adapted from: Fuentes *et al*., 2013; Sinicrope & Dannenberg, 2011)

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Although the aetiology and molecular mechanisms linking the MetS and cancer are still poorly understood, it was proposed that the effects of obesity and IR influence the bioavailability of sex hormones, growth factors and inflammatory markers (Figure 1.3) (Donohoe *et al*., 2011; Mendonça *et al.*, 2015).

Insulin resistance, characterized by hyperinsulinaemia, may predispose individuals with the MetS to develop cancer *via* direct effects of high insulin and increased IGF-1 levels (Arcidiacono *et al*., 2012). In addition, chronic low-grade inflammation, as a result of excess adipose tissue, causes a dysregulation in the synthesis of various adipokines and their actions which have been implicated in the development of cancer (Zhao & Liu, 2013).

Several components of the MetS have also been shown to influence the synthesis and bioavailability of endogenous sex hormones (Lin *et al*., 2013). Adipose tissue distribution, especially android and gynoid body shape may be an underlying factor in the pathophysiology of the MetS and cancer development (Figure 1.3). For example, adipose tissue distribution plays a role in the development of IR, influences growth factor, sex hormones and inflammatory profiles, with android body shape often being associated with a more dysfunctional profile (Alwachi *et al*., 2013; Orbetzova *et al*., 2012; Preis *et al*., 2010; Weinberg *et al*., 2006). Sex hormones, growth factors and inflammatory markers regulate major pathways involved in angiogenesis, cell-proliferation and apoptosis, and thus have the ability to influence carcinogenesis. Therefore, these factors may play a role in the complex pathophysiology of the MetS and risk of cancer development (Sinicrope & Dannenberg, 2011). Some of these factors will be discussed in the following sections.

1.3.3 Role of insulin and insulin-like growth factor-1 (IGF-1)

1.3.3.1 Introduction

Evidence linking growth factors such as insulin, IGF-1 with cancer development; have been proposed by several studies (Arcidiacono *et al*., 2012; Renehan *et al*., 2006; Vucenik & Stains, 2012). Such evidence points to the effect of increased insulin and IGF-1 levels on the activation of tyrosine kinase activity and its downstream signalling pathways (Arcidiacono *et al*., 2012). These growth factors are thought to be underlying factors in the multifaceted pathophysiology of the MetS and cancer development (Vucenik & Stains, 2012).

1.3.3.2 Insulin-IGF axis

The insulin-IGF axis is a complex molecular system that includes three ligands (insulin, IGF-1, and IGF- 2), each with its own receptors and six IGF-binding proteins (IGF-BP-1-6) (Djiogue *et al*., 2013; Roberts *et al*., 2010).

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Insulin-like growth factor-1 is described as a peptide hormone that has hormonal and growth factor properties (Brahmkhatri *et al*., 2015; Djiogue *et al*., 2013). It plays a role in regulating cellular proliferation in response to available energy and nutrient status from diet and body reserves (Brahmkhatri *et al*., 2015; Djiogue *et al*., 2013). Furthermore, IGF-1 regulates growth, development and metabolism of various tissues (Hursting & Hursting, 2012; Hursting *et al*., 2012). Insulin-like growth factor-1 is produced by several tissue types, but is predominantly (70 %) produced by the liver (Friedrich *et al*., 2013; Hursting *et al*., 2012). The production of IGF-1 is subject to both hormonal and nutritional factors (Brahmkhatri *et al*., 2015), while, its secretion is dependent on growth hormone (GH) signalling, mediated by the GH-receptor (Hursting *et al*., 2012). Recently, hyperinsulinaemia and hyperglycaemia have been shown to stimulate IGF-1 production (Ramos-Nino, 2013). Of the two insulin growth factors, IGF-1 mediates GH functions, which in turns promotes cellular proliferation, differentiation and protein synthesis (Friedrich *et al*., 2013). Insulin-like growth factor-2 on the other hand, is a foetal growth factor, but it is also found in small amounts in adult tissues (Friedrich *et al*., 2013). Insulin itself has a range of metabolic functions; however it also has the ability to function as a growth factor with strong mitogenic capacity (Suba, 2012).

Insulin-like growth factor-1 and IGF-2 are both found in the circulation bound to IGF-BP (Renehan *et al*., 2006). Insulin-like growth factor-1 circulates at a high concentration (150-400 ng/ml) in plasma, but mostly in this bound state (Brahmkhatri *et al*., 2015). For example, only 0.5-1.0 % of IGF-1 is "free" in circulation, while the remaining 99 % is bound to IGF-BP (Friedrich *et al*., 2013). Insulin-like growth factor-binding protein is predominately synthesised in the liver by hepatocytes to enhance half-life, regulate bioavailability and offer protection against degradation of IGF-1 and IGF-2 (Saydah *et al*., 2009). The synthesis of IGF-BP is inhibited by insulin and can be stimulated by glucagon, oestrogen and pro-inflammatory cytokines (Lewitt *et al*., 2014). Insulin-like growth factor-binding protein 3 is the most abundant form found in circulation, i.e. approximately 80 % of IGF-1 is bound to IGF-BP3 (Friedrich *et al*., 2013; Lewitt *et al*., 2014). Changes in the concentration of IGF-BP may therefore directly impact the concentration of IGF-1 in circulation (Saydah *et al*., 2009).

1.3.3.3 Insulin-IGF axis and disease states

The insulin-IGF axis has been implicated in the pathophysiology of obesity, IR, T2DM and the MetS (O' Neill & O'Driscoll, 2015). Recently, this axis has been implicated in the development of cancer, as it regulates major pathways involved in proliferation and apoptosis, thus having the ability to influence carcinogenesis (Brahmkhatri *et al*., 2015; Lewitt *et al*., 2014).

Obesity, specifically android body shape-associated obesity, is well-known to contribute to the development of IR, characterized by hyperinsulinaemia and impaired glucose tolerance, i.e. a high BMI, WC and WHR correlates with serum insulin levels, hyperglycaemia, hyperinsulinaemia and T2DM (Djiogue *et al*., 2013; Harvey *et al*., 2011; Orbetzova *et al*., 2012; Ramos-Nino, 2013). It is

proposed that hyperinsulinaemia may predispose individuals with the MetS to develop cancer *via* direct effects of high insulin levels (Westley & May, 2013).

Substantial evidence links hyperinsulinaemia with cancer (Westley & May, 2013). For example, T2DM is associated with an increased risk for rectal, endometrium, pancreatic, breast and prostate cancer (Iyengar *et al*., 2015). Increased levels of plasma C-peptide, a marker of insulin secretion has also been associated with an increased risk for several cancer types (Michaud *et al*., 2007; Verheus *et al*., 2006). In addition, the risk for colorectal, liver, pancreatic and breast cancer has been shown to be even higher in patients who are IR (Arcidiacono *et al*., 2012).

Multiple signalling pathways may be involved in chronic hyperinsulinaemia's role in cancer progression, which can lead to mitogenic, anti-apoptotic and pro-angiogenic effects (Figure 1.4) (Arcidiacono *et al*., 2012). Several studies have shown that high levels of insulin are associated with an increased risk for endometrial, renal, colon and breast cancer (pre- and post-menopausal) (Hursting & Hursting, 2012; Hursting *et al*., 2012), and it is thought that insulin acts as a cancer promoter directly *via* its insulin receptor (ISR) (Roberts *et al*., 2010) (Figure 1.4). More specifically, the activating mitogen activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) pathways may play a role here (Harvey *et al*., 2011). Insulin also plays a role in IGF-1 production which has multiple effects on tumour growth and metastasis (Arcidiacono *et al*., 2012).

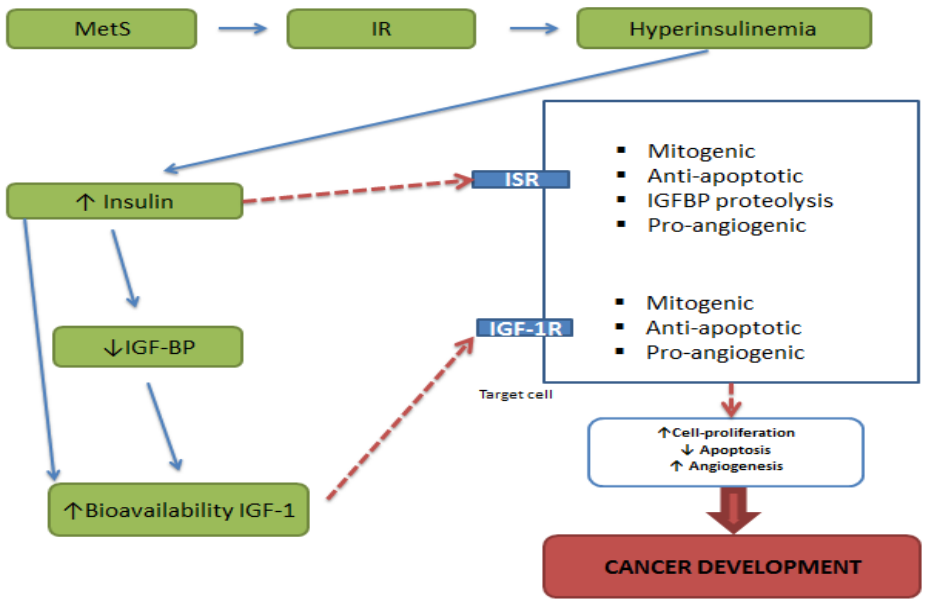

Figure 1.4: Proposed mechanisms linking insulin and IGF-1 to cancer.
(Adapted from Renehan *et al*., 2006; Roberts *et al*., 2010; Rowlands *et al*., 2012)

It is hypothesised that hyperinsulinaemia leads to an increase in the bioavailability of IGF-1 mainly by two mechanisms (Figure 1.4). First, by directly up-regulating IGF-1 production in the liver (Hursting *et al*., 2012). Hyperinsulinaemia, in the hepatic portal circulation, leads to an increase in GH receptor expression, thereby leading to an increase in IGF-1 production (Westley & May, 2013). In addition,

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insulin has a low binding affinity for Insulin-like growth factor-1-receptor (IGF-1R), however, high insulin levels found in obese (android obesity), hyperinsulinaemic and MetS individuals, might bind to and stimulate IGF-1R (Westley & May, 2013). The second mechanism involves indirectly down-regulating the production of IGF-BP by reducing hepatic gene expression and protein synthesis of IGF-BP (Arcidiacono *et al*., 2012; Renehan *et al*., 2006). This leads to an increased bioavailability of IGF-1, which is then free to bind to IGF-1R (Mendonça *et al*., 2015; Ramos-Nino, 2013; Roberts *et al*., 2010).

Insulin and IGF-1 binds to their respective cell surface receptors and thereby activates the PI3K (anti-apoptotic) and/or the MAPK (mitogenic) pathway (Iyengar *et al*., 2015), both of which are primary regulators of cell growth and mitogenesis (Hursting *et al*., 2012). Overstimulation and the activation of MAPK and protein kinase B (PKB) pathways ultimately result in a cellular environment which leads to tumour development (Ramos-Nino, 2013; Roberts *et al*., 2010). Thus, insulin can directly and indirectly stimulate tumour development, and this is likely mediated by IGF-1's mitogenic and anti-apoptotic effects (Arcidiacono *et al*., 2012).

Obesity and the MetS are associated with dysregulation of circulating insulin and IGF-1 levels (Friedrich *et al*., 2012; van Bunderen *et al*., 2013). Several studies found that IGF-1, IGF-2 and insulin levels are increased in the MetS (Friedrich *et al*., 2013; Renehan *et al*., 2006; Roberts *et al*., 2011). Increased circulatory levels of IGF-1 have also been associated with an increased risk for various cancer types, i.e. colorectal, prostate and post-menopausal breast cancer, as well as a poor prognosis (Rowlands *et al*., 2012). In addition, calorie restriction reduces IGF-1 as well as insulin levels (Vucenik & Stains, 2012).

It may be possible that an increase in both insulin and IGF-1 may activate the overstimulation of ISR and IGF-1R which leads to the activation of cell proliferation, an increase in protein synthesis and a decrease in apoptosis (Braun *et al*., 2011). Evidence also showed that the MetS is related to an overstimulation of ISR and IGF-1R (Arcidiacono *et al*., 2012). Although insulin and IGF-1 may play a role in cancer development and progression (Mendonça *et al*., 2015), other factors also need to be investigated for a clearer understanding of the complex interaction between the MetS and cancer.

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1.3.4. The Role of adipose tissue and inflammatory biomarkers

1.3.4.1 Introduction

The role of inflammation as a primary factor in the pathophysiology of various disease states, i.e. IR, T2DM, CVD, obesity and cancer has been studied (Cefalu, 2009; Shrivastava *et al*., 2015). Recent evidence points to the fact that chronic low-grade inflammation, as a result of excess adipose tissue, might be implicated in the development of obesity-related metabolic diseases (Jung & Choi, 2014).

1.3.4.2 Adipose tissue and inflammation

As a metabolic active endocrine organ, adipose tissue secretes various bioactive substances known as adipokines, which have both pro- and anti-inflammatory properties (Mendonça *et al.*, 2015; Zhao & Liu, 2013). These include, but are not limited to, C-reactive protein (CRP), leptin, adiponectin (Apn), tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), resistin and angiotensinogen (Gade *et al*., 2010). These adipokines have diverse roles in food intake regulation, glucose and lipid metabolism, insulin sensitivity, adipocyte differentiation, inflammation, coagulation and blood pressure regulation (Fuentes *et al*., 2013). Dysregulation in the synthesis of these adipokines and their actions, in relation to obesity, have been linked to the development of various disease states, including the MetS, T2DM and cancer (Zhao & Liu, 2013).

Acute inflammation is characterized by calor, dolor, erythema and leukocyte infiltration (Kushner *et al*., 2010). However, inflammation is also described as an increase in the concentration of pro- and anti-inflammatory cytokines, as well as acute phase proteins (Kushner *et al*., 2010). A poorly defined expression, low-grade inflammation, has been used in the literature to describe conditions in which the classical symptoms of inflammation are absent (Brenner *et al*., 2014). These include obesity, IR and the MetS (Kushner *et al*., 2010). Obesity is characterized by low-grade chronic inflammation due to a deregulated adipokine profile, and increased secretion of pro-inflammatory cytokines and their related signalling pathways (Jung & Choi, 2014). Adipose tissue of obese individuals is characterized by an infiltration of immune cells i.e. lymphocytes and macrophages. Furthermore, a phenotypic switch from anti-inflammatory (M2) to pro-inflammatory macrophages (M1) also occurs, which are all hallmarks of a pro-inflammatory state, thereby linking obesity to systemic inflammation (Figure 1.5) (Fuentes *et al*., 2013).

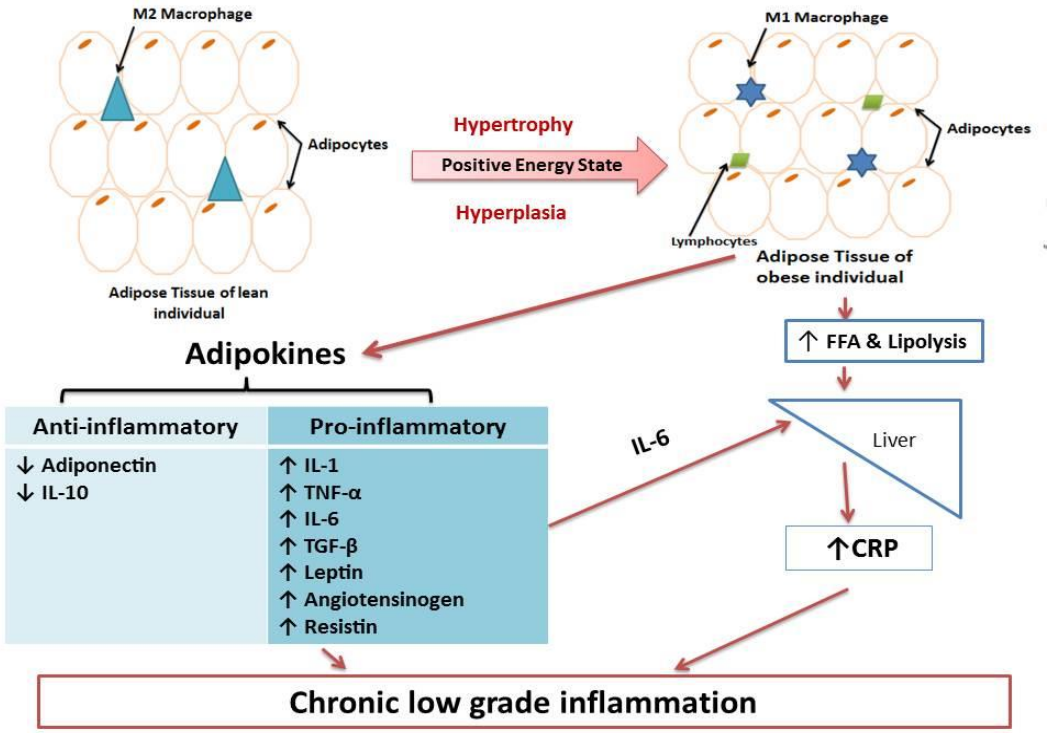

Figure 1.5: The proposed link between obesity and inflammation.
(Adapted from: Donohoe *et al*., 2011)

During a positive energy state, adipose tissue stores excess energy as TG in the lipid droplets of adipocytes, which leads to hyperplasia, as well as hypertrophy (Figure 1.5) (Bjørndal *et al*., 2011; Jung & Choi, 2014). The number of adipocytes has been genetically pre-determined, and an increase in fat mass in adulthood is thought to be mainly due to hypertrophy (Jung & Choi, 2014), resulting in the deregulated secretion of various adipokines (Lasselin *et al*., 2014). Adipocyte hypertrophy leads to an increased production of pro-inflammatory cytokines, namely TNF-α, IL-1β, IL-6, transforming growth factor beta (TGF-β), leptin, acute phase proteins (hepatic CRP) and a decrease in Apn, which has anti-inflammatory properties (Fain, 2006). In addition, adipose tissue itself can also secrete CRP (Ikeoka *et al*., 2010). As a result, a pro-and anti-inflammatory imbalance occurs, which leads to a low-grade inflammatory state (Fuentes *et al*., 2013; Zhao & Liu, 2013). Additionally, adipose tissue hypertrophy leads to the redirection of fatty acids to the liver; promoting dyslipidaemia, (increased free fatty acids (FFA), TG and LDL-c and decreased HDL-c), and increased lipolysis, all of which contributes to the development of low-grade inflammation (Bjørndal *et al*., 2011; Jung & Choi, 2014).

It is clear that the pathophysiology behind obesity-associated inflammation includes a network of cells, pathways and adipokines (Brenner *et al*., 2014). In addition to adipose tissue hyperplasia, hypertrophy, immune cell infiltration, other factors have also been proposed to play a role in low-grade inflammation (Balistreri *et al*., 2010). These include the role of adipocyte differentiation, induced by increased levels of pro-inflammatory adipokines, which in turn leads to the secretion of more pro-inflammatory cytokines (Pérez-Hernández *et al*., 2014). In addition, hypoxia induced by the

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rapid expansion of adipose tissue, also induces the release of inflammatory mediators, thus further contributing to inflammation (Trayhurn, 2013). Therefore, although adipose tissue remodelling, differentiation and hypoxia underline the complexity of obesity-associated inflammation, the exact mechanism still needs to be elucidated.

1.3.4.3 Role of inflammatory markers

Various adipokines, cytokines, chemokines, hormones and growth factors are thought to play a role in the MetS, and the association with lifestyle-associated cancer risk (Cefalu, 2009). Adipose tissues from patients that have the MetS display a deregulated adipokine and cytokine profile, which is shifted towards a chronic inflammatory state (Maury & Brichard, 2010).

1.3.4.3.1 C-reactive protein (CRP)

C-reactive protein forms part of the non-specific acute phase immunological response to inflammation, infection and tissue damage (Braun *et al*, 2011; Ingle & Patel, 2011). During the acute phase response, numerous proteins are rapidly synthesised by hepatocytes under the control of pro-inflammatory cytokines, such as IL-1, IL-6 and TNF-α (Ingle & Patel, 2011; Pepys & Hirschfield, 2003). C-reactive protein is a direct measurement of this acute phase, and is therefore considered as a measure of inflammation as its levels rapidly rise in response to it (Aguiar *et al*., 2013).

C-reactive protein is the most studied and characterized systemic inflammatory biomarker used in epidemiological studies (Braun *et al*, 2011), which have shown that increased CRP levels are associated with a variety of metabolic (CVD and T2DM), inflammatory and chronic conditions (Brenner *et al*., 2014; Kushner *et al*., 2010; Pepys & Hirschfield, 2003). In addition, CRP is also related to obesity, since obesity is known as a low-grade chronic inflammatory condition (Braun *et al*, 2011).

The association between obesity and CRP has been extensively studied (Choi *et al*., 2013; Illán-Gómez *et al*., 2012; Kao *et al*., 2009). One specific study showed that 35 % of men, and 60 % of women with a BMI of more than 30 kg/m^2 showed increased CRP levels, indicating a possible correlation between CRP and the volume of adipose tissue in the body (Kao *et al*., 2009). Alwachi *et al*. (2013) specifically showed that the mean high sensitivity CRP (hsCRP) levels were significantly higher in post-menopausal women with an android body shape (5.39 mg/L) when compared to post-menopausal women with a gynoid body shape (2.51 mg/L) ($p < 0.05$), suggesting that adipose tissue distribution may also be associated with increased CRP levels low-grade inflammation (Alwachi *et al*., 2013). More specifically related to the MetS, it was proposed to add as a component of the MetS definition (Ridker *et al*., 2004), since CRP has been shown to correlate with individual components of the MetS, such as abdominal obesity, hyperinsulinaemia, IR and dyslipidaemia (da Cruz *et al*., 2013).

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An increase in CRP has been found in patients with the MetS (den Engelsen *et al*., 2012). The study by den Engelsen *et al*. (2012) found a higher median hsCRP of 2.2 mg/L (inter quartile range (IQR) 1.2-4.0) in MetS participants when compared to participants without the MetS (1.7 mg/L (IQR 1.0-3.4)). In addition, an increase in the number of the MetS components was associated with an increase in median hsCRP levels, and the presence of the MetS, WC, TG and HDL-c all showed a significant trends with increasing hsCRP categories (den Engelsen *et al*., 2012), implying that the MetS could play a role in low-grade inflammation. Similar results of higher CRP levels with more MetS components were evident in the studies by Mahajan *et al*. (2012), Sur *et al.* (2014) and Vidyasagar *et al.* (2013).

Vidyasagar *et al.* (2013) also showed higher CRP levels in MetS patients with abdominal obesity compared to those without abdominal obesity (1.4 mg/L *vs* 0.8 mg/L), an effect which was age-dependent. Different obesity indices (WHR, WC and TBF %) are also associated with higher CRP levels in both men and women with the MetS (Lin *et al.*, 2010). After adjusting for hyperglycaemia, hypertension, hypertriglyceridemia and low HDL-c, only TBF % remained significant, which led to the conclusion that TBF % was a better predictor of inflammation when compared to WC, WHR and BMI. This study was however limited by its cross-sectional study design, as well as the population studied, which again highlights the need for different obesity cut-off values for different ethnic groups (Alberti *et al*., 2009; IDF, 2006).

Although numerous studies have focussed on CRP and the MetS, the specific mechanism involved is still unknown; however, it is thought to be mediated by adipose tissue dysfunction predominantly (Lin *et al*., 2010). The role of CRP in the pathophysiology of the MetS may be due to one of the following mechanisms: Firstly, CRP may increase blood pressure by decreasing nitric oxide synthase and by triggering cardiac fibrosis which can cause vasoconstriction (Schwartz *et al*., 2007; Zhang *et al*., 2010[A]). Secondly, increased CRP has also been associated with IR by increasing ISR substrate phosphorylation. This may lead to deregulated insulin-stimulated uptake and glucose transporter type four translocation, which can cause impaired insulin sensitivity (Devaraj *et al*., 2009). Lastly, CRP could also directly impair insulin synthesis *via* CRP-mediated inflammation and the production of reactive oxygen species (ROS) (Brenner *et al*., 2014).

While clear evidence links CRP to the development of the MetS and/or its components, there remains little support linking CRP, the MetS and cancer development. Epidemiological studies have shown that CRP levels, measured by high sensitivity assays, do not only reflect the presence of cancer, but it may be associated with an increased risk for future development of cancer in apparently healthy individuals (Allin & Nordestgaard, 2011). Increased CRP levels have been observed in patients with pancreatic, oesophageal, colorectal, breast, lung, endometrium, ovarian and prostate cancer, and was

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also associated with a poor prognosis (Allin & Nordestgaard, 2011; Brenner *et al*., 2014; Guo *et al*., 2015).

It is currently unknown whether increased CRP levels play a causal role in the pathophysiology of the MetS and its link to cancer, if it is just a biomarker to identify individuals with a greater risk, or if it is associated with the prognosis of several cancer types (Allin & Nordestgaard, 2011). However, although other inflammatory markers may potentially play a similar role in cancer development, which, include pro- and anti-inflammatory cytokines, as well as adipokines lepin and Apn, these markers were not the focus of this thesis.

1.3.4.4 Proposed link between MetS, inflammation and cancer development

Substantial evidence links chronic inflammation and the risk of cancer development (Figure 1.6) (Lumeng & Saltiel, 2011; Pérez-Hernández *et al*., 2014; Rodríguez *et al*., 2013). Obesity and the MetS is characterized by low-grade inflammation, primarily mediated by hypertrophy, hyperplasia and immune cell infiltration, ultimately leading to an deregulated synthesis of several adipokines (Cefalu, 2009; Lasselin *et al*., 2014; Maury & Brichard, 2010). This includes an increase in pro-inflammatory cytokines and acute phase proteins, as well as a decrease in anti-inflammatory cytokines (Maury & Brichard, 2010).

The first link between the MetS, inflammation and cancer development involves several inflammatory mediators (IL-6 and TNF-α) as they have been found to correlate to tumour development and progression (Pérez-Hernández *et al*., 2014), possibly as a result of the downstream effects they may have on major pathways involved in angiogenesis, cell-proliferation and apoptosis, thus having the ability to influence carcinogenesis (Braun *et al*., 2011; Guo *et al*., 2015) (Figure 1.6). In addition, other cancer-causing mechanisms which include loss of tumour suppressor function, increased cell cycling and stimulation of oncogenes, can also be related to cytokines and their sustained inflammatory signalling pathways (Landskron *et al*., 2014).

Leptin and Apn also have a potential role in the risk of cancer development. Leptin has been shown to play a role in inflammation by increasing the synthesis of pro-inflammatory cytokines, and a role in cancer development by increasing cell proliferation and angiogenesis (Rodríguez *et al*., 2013). This is supported by elevated leptin levels found in cancer patients (Aleksandrova *et al*., 2012). Adiponectins' role in the risk of cancer development can be explained by its ability to increase apoptosis (Mendonça *et al*., 2015). Adiponectin levels are decreased in patients with obesity, the MetS and cancer (Gnacińska *et al*., 2010; Otake *et al*., 2010; Zhao & Liu, 2013).

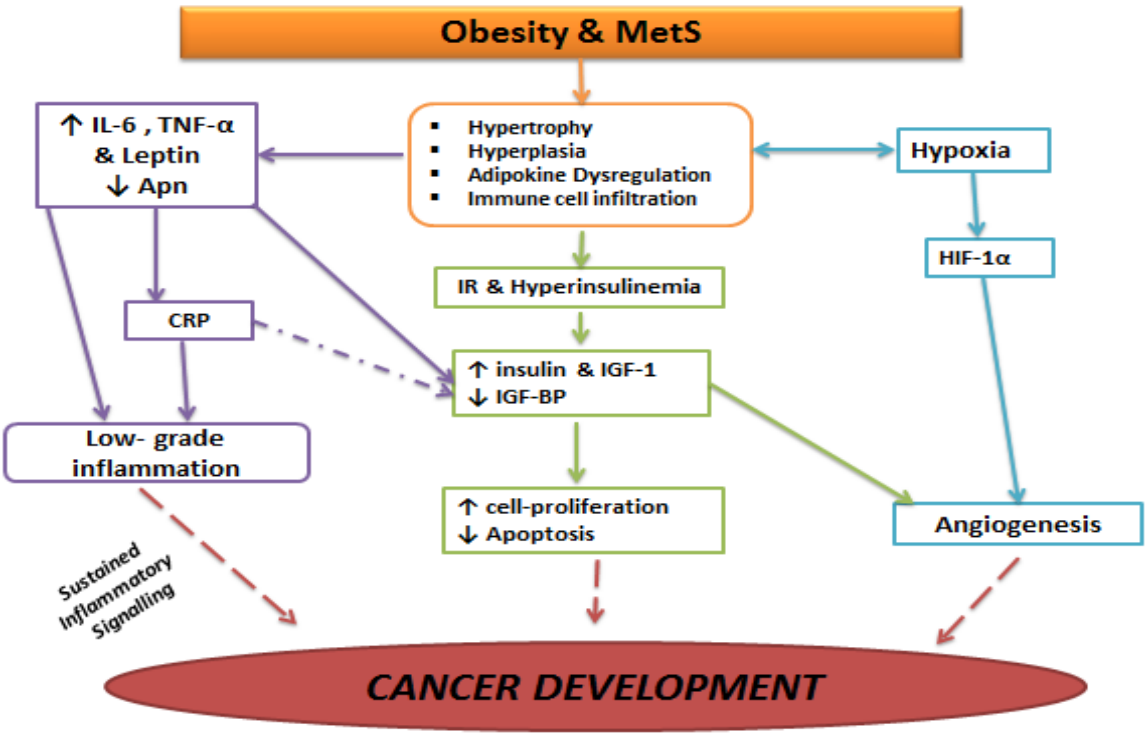

Figure 1.6: The proposed link between metabolic syndrome, inflammation and cancer development.
(Adapted from: Lumeng & Saltiel, 2011; Pérez-Hernández *et al*., 2014)

Secondly, inflammation may be linked to cancer development *via* the insulin-IGF-axis (Figure 1.6). In addition to cytokines regulating CRP synthesis in the liver, cytokines also has effects on the synthesis of IGF-1 (Lumeng & Saltiel, 2011). It has been shown that cytokines regulate IGF-1 synthesis by either influencing GH synthesis or resistance (Lumeng & Saltiel, 2011). Thus, it may be possible that other inflammatory mediators, such as CRP, may also have an influence on IGF-1 synthesis, since it has been shown to potentially cause impaired insulin sensitivity, by affecting ISR substrate phosphorylation (Devaraj *et al*., 2009). For example, a study by Kaushal *et al*. (2004) found that participants with the MetS had lower IGF-1BP levels (14.4 µg/l *vs* 25.4 µg/l, $p<0.001$) and higher CRP (1.9 mg/L *vs* 1.0 mg/L, $p<0.001$) levels *vs* participants without the MetS. A negative correlation between CRP and IGF-1 ($r=-0.11$, $p<0.001$) was also evident (Kaushal *et al*., 2004), whereas Colangelo *et al*. (2009) showed a positive correlation. Since, evidence links hyperinsulinaemia with cancer development, it may be possible that an increase in CRP concentration levels may have an effect on the synthesis of IGF-1, thereby indirectly contributing to cancer development (Colangelo *et al*., 2009; Kaushal *et al*., 2004).

In addition, during rapid adipose tissue expansion, hypoxia is induced (Figure 1.6) (Trayhurn, 2013). Adipose tissue in response to hypoxia synthesises hypoxia inducible factor-1α (HIF-1α) (Trayhurn, 2013). Hypoxia inducible factor-1α binds to transcription factors on vascular endothelial growth factor (VEGF) and angiopoietin-2 genes and thereby inducing angiogenesis, thus contributing to cancer development (Trayhurn, 2013).

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Lastly, human adipocytes express sex steroid hormone receptors, whereby oestrogens can stimulate the proliferation of pre-adipocytes, potentially contributing to obesity-associated inflammation (Suba, 2012). Also, oestrogen-containing medications have an effect on CRP levels (Kushner *et al*., 2010).

1.3.5 Role of sex hormones

1.3.5.1 Introduction

Epidemiological studies provide evidence implicating sex steroid hormones as a causal factor in the pathophysiology of the MetS and risk of cancer development (Iyengar *et al*., 2015; Zhu *et al*., 2010). Different components of the MetS have been associated with a deregulated sex steroid hormone profile, characterized by excessive androgen and oestrogen synthesis (Brand *et al*., 2011; Suba, 2012). A deregulated sex hormone profile has also been associated with the development and progression of various cancers types, including breast, ovarian and colon cancer (Floreani *et al*., 2002; Key *et al*., 2011; Lin *et al*., 2013).

1.3.5.2 Oestrogens and androgens

Endogenous sex hormones can be classified into three steroid groups; oestrogens, androgens and progesterone's (Kim & Halter, 2014). Oestrogen is known as the primary female sex steroid hormone having several endogenous forms, namely, oestrone (E1), oestradiol (E2), and oestriol, with E2 being the major physiological form (Pradhan, 2014). Oestrogen is responsible for the development and the maintenance of female gender characteristics (Folkerd & Dowset, 2010). In women, oestrogen is predominantly produced by the ovaries; however it can also be produced by peripheral tissues (Suba, 2012). In post-menopausal women, the conversion of androgens to oestrogens can also occur in peripheral tissues, i.e. adipose, breast, brain, endometrium, bone, endothelium, muscle and aortic smooth muscle cells (Calle & Kaaks, 2004; Iyengar *et al*., 2013; Suba, 2012). Most of these tissues, specifically breast and endometrial tissue, have a high demand for oestrogen (Suba, 2012), and it is crucial that the production of sex hormones by peripheral tissues helps to preserve structural integrity, as well as its functional activity (Calle & Kaaks, 2004; Suba, 2012).

Adipose tissue plays an endocrinological role in the synthesis of sex hormones as it contains enzymes involved in the biosynthesis of these sex hormones (Suba, 2012; Westley & May, 2013). Adipocytes express 17β-hydroxyl steroid dehydrogenase (17β-HSD), an enzyme responsible for the conversion of Δ4-androstenedione (ΔA4) to E2, while aromatase, a P450 cytochrome enzyme, catalyses the final step of oestrogen synthesis by aromatisation of androgens to oestrogens, i.e. testosterone (T) is converted to oestrone (E1) (Westley & May, 2013) (Figure 1.7).

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Oestrogen has been associated with all the individual components of the MetS (Pradhan, 2014). Abdominal obesity is a primary component of the MetS and may be influenced by, the sex hormone profile. Oestrogen, for example, plays an important role in fat distribution (Momesso *et al*., 2011). A normal oestrogen profile in women is associated with gluteo-femoral fat deposition (gynoid body shape), while androgen excess and oestrogen deficiency is associated with visceral obesity and android body shape in both pre-and post-menopausal women, which in turn increases the risk for CVD and the MetS (Momesso *et al*., 2011; Orbetzova *et al*., 2012). In addition, oestrogen has a regulatory role in the differentiation and metabolism of adipocytes (Suba, 2012).

Oestrogen may play a role in maintaining blood lipid levels, as shown in a study by Lin *et al.* (2013). Post-menopausal women have elevated levels of TC, LDL-c and TG and decreased HDL-c levels *vs* pre-menopausal women (Lin *et al*., 2013). Oestrogen has also been associated with hypertension, IR and, is also implicated in the regulation of insulin production capacity of pancreatic beta-cells (Suba, 2012; Subramanian *et al*., 2011). Thus, changes in oestrogen levels in the body may influence the development of the MetS, or its components (Brand *et al*., 2011; Suba, 2012).

Although testosterone is predominantly found in the testes of men (Al kindi *et al*., 2012), the adrenal glands are also capable of producing T in small quantities (Folkerd & Dowset, 2010). In women, T is produced by both the ovaries and the adrenal glands, however at a much lower concentration (Folkerd & Dowset, 2010). Over secretion of androgen (increased T, and SHBG Levels) has been observed in women with the MetS (Weinberg *et al*., 2006), and is also related to the individual components of the MetS itself (Suba, 2012).

1.3.5.3 Sex hormone binding globulin (SHBG)

Sex hormones circulate in the blood mainly in three forms; (i) bound to SHBG, (ii) bound to albumin or other plasma proteins or, (iii) in a free (unbound) state (Hammond *et al*., 2012).

Sex hormone binding globulin' functions include transport of sex steroids, regulating free (unbound) hormone concentration and their transport to target tissues (Avvakumov *et al*., 2010; Hammond *et al*., 2012). Each SHBG consist of two steroid binding sites (Avvakumov *et al*., 2010), and research have shown that none, one or both binding sites may be occupied by the same or different sex steroid hormone simultaneously (Hammond *et al*., 2012). Testosterone has a much higher binding affinity for SHBG compared to E2 (Wallace *et al*., 2013). Sex hormone binding globulin levels also differ according to gender, due to occupancy of its binding sites (Wallace *et al*., 2013). This is evident in women, whereas, only a small fraction of SHBG binding sites are occupied by sex steroids, whereas in men it is mostly occupied by T (Hammond *et al*., 2012). According to the free sex hormone hypothesis, only sex hormones that are in the free (unbound) state are thought to be biologically

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active, and since they have lipophilic properties, they have the ability to passively diffuse into target cells (Rosner, 2006).

Measurement of SHBG, for example, is an indirect measurement of sex hormone excess, with its concentrations mainly related to the levels of free oestrogens and androgens (Kalyani *et al*., 2009). The free androgen index (FAI) is a ratio used to estimate the androgen status of both men and women (Al kindi *et al*., 2012), and it is calculated as the percentage ratio of total testosterone to SHBG concentration (Hammond *et al*., 2012). A high FAI has been associated with the MetS, and several individual components of the MetS in female participants (Folkerd & Dowset, 2010).

1.3.5.4 Evidence of the MetS, sex hormone and SHBG alterations

Components of the MetS have been shown to have an influence on the synthesis and the bioavailability of endogenous sex hormones, i.e. oestrogens and androgens (Brand *et al*., 2011). An increase in oestrogen and androgen production has been found in overweight and obese women due to excess adipose tissue after menopause (Kalyani *et al*., 2009; Weinberg *et al*., 2006). In addition, obesity has also been associated with an increase in tissue level of both E1 and E2 (Westley & May, 2013).

Kalyani *et al*. (2009) showed that high T and oestrogen levels in women are associated with a higher incidence of T2DM. Women who developed T2DM had much higher levels of T and total E2 compared to those who didn't develop T2DM after follow-up (Kalyani *et al*., 2009). Higher total E2, T and FAI, together with lower SHBG levels were found in women who had the MetS compared to controls (Weinberg *et al*., 2006). Higher total E2 levels were also associated with a higher BMI and abnormal glucose homeostasis, while low SHBG levels and high FAI was associated with all the components of the MetS (i.e. obesity, low HDL-c, high TC, high TG levels, hypertension and abnormal glucose metabolism) in post-menopausal women (Weinberg *et al*., 2006). One specific study also showed that post-menopausal women with the MetS had characteristics of hyperandrogenism (higher T and lower SHBG levels) when compared to post-menopausal women without the MetS (Ziaei & Mohseni, 2013). Results from these studies all concluded that the association between the MetS, or the MetS components, with the bioavailability of endogenous sex hormones may likely be due to adiposity and IR (Kalyani *et al*., 2009; Weinberg *et al*., 2006). This is plausible since IR, hyperinsulinaemia and high IGF-1 can lead to defective oestrogen production by inhibiting aromatase enzyme gene expression (Suba, 2012).

Evidence suggests that SHBG may offer more biological significance in addition to its transport function (Folkerd & Dowset, 2010). Low serum SHBG levels have been found in patients with T2DM and have been associated with an increased risk for T2DM (Ding *et al*., 2009; Kalyani *et al*., 2009; Weinberg *et al*., 2006). Obesity, hyperinsulinaemia and high IGF-1 levels have also been associated

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with a decrease in SHBG production, thus leading to an increase in oestrogen levels (Braun *et al*., 2011; Westley & May, 2013). These associations have been found to be casual and likely to be mediated by effects of IR (Kalyani *et al*., 2009; Suba, 2012; Weinberg *et al*., 2006).

The relationship between IR and low SHBG levels is stronger in women compared to men, and even stronger in post-menopausal women (Wallace *et al*., 2013). Two studies have shown that SHBG levels decrease in post-menopausal women (Folkerd & Dowset, 2010; Wallace *et al*., 2013), and that low levels of SHBG are associated with an androgen excess (Folkerd & Dowset, 2010). In addition, both hyperinsulinaemia and IR have been associated with hyperandrogenism in women (Suba, 2012). In pre-menopausal women on the other hand, low SHBG levels are usually linked to polycystic ovarian syndrome (Simó *et al*., 2015). More specifically, Korhonen *et al*. (2003) showed that pre-menopausal women with the MetS had increased T, decreased SHBG levels and increased FAI, typical to that of polycystic ovarian syndrome (Korhonen *et al*., 2003)

It may be possible that IR has an effect on hepatic synthesis of SHBG (Simó *et al*., 2015). However, the evidence supporting the association between IR and changes in sex hormone levels are largely circumstantial. Sex hormone binding globulin levels are low in most cases of hyperinsulinaemic patients; however most of these patients also have hyperglycaemia and present with other metabolic alterations that have the ability to alter transcription factors which regulates SHBG expression in the liver (Hammond *et al*., 2012).

Multiple other factors may confound the association between IR and SHBG levels, such as menopausal status, age, gender, sex steroid concentration, obesity and body composition (Wallace *et al*., 2013). Body composition (in particular the percentage body fat) is a main determination factor for SHBG levels (Hammond *et al*., 2012). For example, high levels of SHBG have been found in anorexic women, being a reflection of a chronic fasted liver state, while obesity has been associated with a decrease in SHBG production (Koskova *et al*., 2009; Westley & May, 2013). This suggests that no single factor can explain this complex association.

1.3.5.5 Proposed mechanisms behind sex hormone's role in the pathophysiology of the MetS and cancer development

Adipose tissue is known to produce endogenous sex hormone metabolising enzymes, namely aromatase, as well as hydroxysteroid dehydrogenase (17β-HSD) (Calle & Kaaks, 2004). These enzymes catalyse the synthesis of oestrogens from androgenic precursors produced by the gonads and adrenal glands (Calle & Kaaks, 2004).

The MetS and its components are associated with increased oestrogen and T production (Brand *et al*., 2011) (Figure 1.7). In overweight and obese individuals, there is an increase in aromatase and 17β-HSD enzyme production. Which, can lead to an increase in the conversion of androgens, i.e. ΔA4 and

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T into oestrogens (E1 and E2) (Brand *et al*., 2011; Braun *et al*, 2011; Mendonça *et al.*, 2015), while, 17β-HSD enzyme catalyses the conversion of ΔA4 into T and E1 into E2 respectively (Calle & Kaaks, 2004).

Oestrogen is further increased by obesity-associated hyperinsulinaemia and IGF-1 overexpression, resulting in a decrease in SHBG produced by the liver (Braun *et al*, 2011). This increase in E2 and T production, in combination with a decrease in SHBG levels, leads to an increase in the bioavailability of E2 and T (Folkerd & Dowset, 2010), which then diffuses to target cells, where they can bind to their respective oestrogen and androgen receptors (Folkerd & Dowset, 2010).

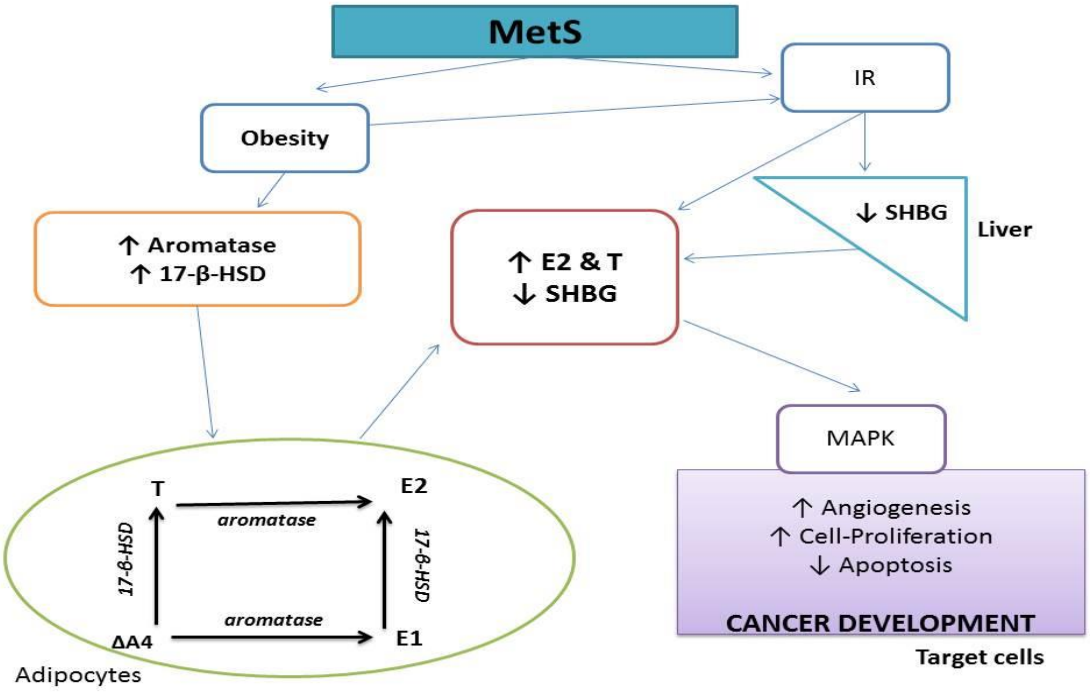

Figure 1.7: The proposed mechanisms by which sex hormones are linked to cancer development.
(Adapted from: Donohoe *et al*., 2011; Folkerd & Dowsett, 2010)

This excessive bioavailability of oestrogen is thought to act through ISR, IGF-1R and oestrogen-receptor pathways, which results in the activation of MAPK pathways (Hursting *et al*., 2012). Mitogen activated protein kinase activation leads to mitogenic, anti-apoptotic and pro-angiogenic effects *via* oestrogen receptor-α antagonism, and ultimately leading to the promotion of cancer development in oestrogen-sensitive tissues (Braun *et al*., 2011; Hursting *et al*., 2012; Westley & May, 2013). Oestrogen-sensitive tissues are overstimulated in obese *vs* lean women; this increases the risk for cancer by stimulating the growth and progression of cancer (Iyengar *et al*., 2013). Oestrogens are known to stimulate the proliferation of epithelial breast cells and exert a mutagenic effect (Iyengar *et al*., 2013). Furthermore, oestrogen can also be metabolised into deoxyribonucleic acid (DNA) reactive metabolites leading to the induction of mutagenesis, i.e. inducing free-radical DNA production, causing genetic instability and mutations in cells (Hursting *et al*., 2012; Roberts *et al*., 2010). In

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addition, inflammatory mediators like IL-6, TNF-α and leptin has also been shown to induce aromatase production which leads to an increase in oestrogen production (Morris *et al*, 2011).

Body composition compartments, especially abdominal obesity, seem to be an underlying factor in the pathophysiology of the MetS and the risk of cancer development. For example, adipose tissue plays a role in oestrogen production; it is a source of low-grade inflammation and plays an important role in the development of IR (Cefalu, 2009; Friedrich *et al*., 2012; Suba, 2012). Since adipose tissue is an important part of the puzzle, body composition will be discussed in the next section.

1.4 Body composition

1.4.1 Introduction

Anthropometry is defined as the study of human body measurements in terms of adipose, muscle and bone tissue dimensions (Stewart & Sutton, 2012). The study of anthropometry includes a variety of different body measurement, i.e. weight, height, recumbent length, skinfold thickness, circumference, limb lengths and breaths using standardized equipment (Eston & Reilly, 2009; Stewart & Sutton, 2012). Anthropometry is also a key contributor of knowledge in terms of nutrition assessment in both adults and children (Eston & Reilly, 2009), and can be used to estimate the different levels of body composition (Cornier *et al*., 2011).

1.4.2 Body composition: measurements, indices and ratios in health assessment

The main rationale of utilizing body composition in a clinical setting is to describe and evaluate changes in the body in relation to nutritional status and lifestyle-related diseases (Thibault *et al*., 2012). The assessment of body composition has been applied in various disciplines including medical science, sport science, anthropology and child growth (Eston & Reilly, 2009; Norton & Olds, 1996; Stewart & Sutton, 2012). Interestingly, body composition does not only provide information regarding disease risk, but also morbidity, mortality, and contributes to improve general health care guidelines (Stewart & Sutton, 2012). Over the last few decades there has been an increase in the number of techniques to assess body composition, specifically adiposity (Stewart & Sutton, 2012), which include BIA and traditional anthropometric assessments (BMI, WC, hip circumference (HC) and WHR).

1.4.2.1 Bio-electrical impedance analysis (BIA)

To date there is no direct measure to assess adipose tissue (Stewart & Sutton, 2012). However, an accurate estimate can be made by means of ultrasound, magnetic resonance imaging (MRI), computed tomography (CT) and dual-energy X-ray absorptiometry (DEXA) techniques (Eston & Reilly, 2009; Rothney *et al*., 2009; Stewart & Sutton, 2012).

Magnetic resonance imaging, CT and DEXA are considered the gold standards for measuring adipose compartments of the abdomen and gluteo-femoral region, however these methods are relatively expensive and complex, and not really practical in a field study setting as it is not portable, and requires high levels of training (Eston & Reilly, 2009; Norton & Olds, 1996; Rothney *et al*., 2009; Stewart & Sutton, 2012), which is not the case with bio-electrical impedance analysis (BIA).

Bio-electrical impedance analysis can be used to quantitatively measure different body composition compartments, including TBF %, fat mass (FM), fat free mass (FFM) and total body water (TBW) in men and women (Figure 1.8) (Stewart & Sutton, 2012; Thibault *et al*., 2012). The use of BIA to estimate body composition in a clinical setting has several advantages; (i) it is easily available, (ii) quick, (iii) portable, (iv) non-expensive, (v) and easy to use with minimal training, (vi) and has also been shown to produce similar results to that seen when using the gold standard techniques (Eston & Reilly, 2009; Stewart & Sutton, 2012; Thibault *et al*., 2012).

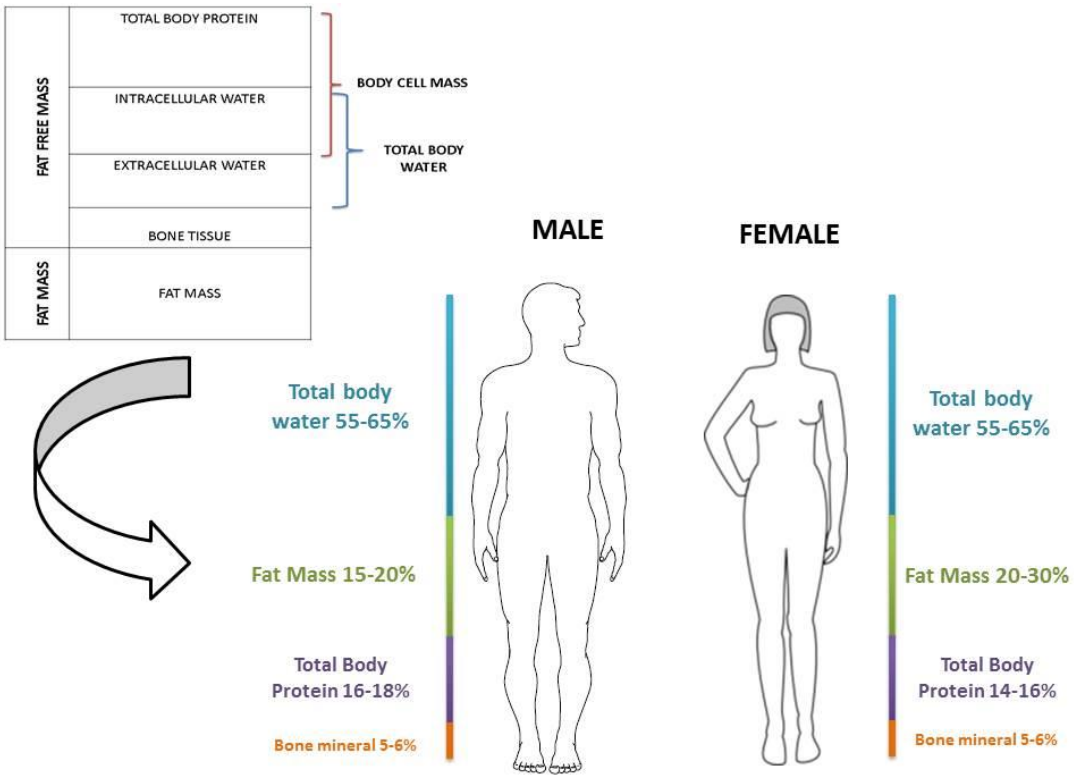

Figure 1.8: Body composition compartments in the human body and estimated reference ranges.
(Adapted from: Eston & Reilly, 2009; Stewart & Sutton, 2012; Thibault *et al*., 2012)

Bio-electrical impedance analysis is based on the principle of electrical impedance, i.e. electrical conductivity characteristics of hydrous (electrolytes) and non-hydrous (cell membrane) components of cells (Eston & Reilly, 2009). This is possible due to the fact that water has a high electrolyte

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content and can easily conduct an electrical current in comparison to adipose tissue which is a poor conductor of electrical current (Eston & Reilly, 2009; Thibault *et al*., 2012).

Although BIA is a useful alternative to use in a clinical setting, most methods come with disadvantages; and BIA is no exception. Various predictive equations based on gender, age, height, weight and ethnicity is applied to estimate FM, FFM and TBW (Dehghan & Merchant, 2008). Bio-electrical impedance analysis equations are population-specific and can only be useful for populations with similar characteristics to those of the reference population (Dehghan & Merchant, 2008; Thibault *et al*., 2012). Thus, these equations cannot be generalized to diverse populations as it will yield inconsistent results (Dehghan & Merchant, 2008).

In addition, despite BIA's ease of use and high reproducibility, BIA measurements are also influenced by the placement of the electrode, dehydration, exercise, temperature and a conductive surface (Eston & Reilly, 2009). Thus, special care should be taken in preparation of study participants and equipment prior to the performance of the BIA test. Despite these limitations, BIA is still a quick, easy and reliable method to estimate different body compartments, and is feasible to use in a field study setting. Various indices and ratios can be determined using anthropometry, the most well-known predictor being the BMI (Cornier *et al*., 2011).

1.4.2.2 Body mass index (BMI)

Different adiposity measurements have been used to categorize individuals as being underweight, normal, overweight or obese (Table 1.5) (Stewart & Sutton, 2012). Apart from BMI, other measurements typically include; WC, HC, WHR and TBF % (Eston & Reilly, 2009; Norton & Olds, 1996; Stewart & Sutton, 2012).

Body mass index (kg/m^2) is an index portraying an individual's weight per unit of height, and is defined as weight in kilograms (kg) divided by the square height in meters (m) (Phillips *et al*., 2013; Stewart & Sutton, 2012).

Although BMI cut-off points are age-dependant, they are similar for men and women (WHO, 1995; WHO, 2000; WHO Expert Consultation, 2004), and may also not correspond to the same degree of body fat in different populations. This is in part due to differences in body composition, i.e. percentage body fat and fat distribution in different populations which, can result in health risks associated with increased BMI. Thus, additional cut-off points have been recommended by the WHO to overcome these difficulties (WHO Expert Consultation, 2004).

Table 1.5: International classification accourding to BMI cut off points for adults

Classification	BMI (kg/m^2)	
	Principal cut-off points	***Additional cut-off points***
Underweight	< 18.50	< 18.50
Severe thinness	< 16.00	< 16.00
Moderate thinness	16.00 - 16.99	16.00 - 16.99
Mild thinness	17.00 - 18.49	17.00 - 18.49
Normal range	18.50 - 24.99	18.50 - 22.99
		23.00 - 24.99
Overweight	≥ 25.00	≥ 25.00
Pre-obese	25.00 - 29.99	25.00 - 27.49
		27.50 - 29.99
Obese	≥ 30.00	≥ 30.00
Obese class I	30.00 - 34.99	30.00 - 32.49
		32.50 - 34.99
Obese class II	35.00 - 39.99	35.00 - 37.49
		37.50 - 39.99
Obese class III	≥ 40.00	≥ 40.00

(Adopted from: WHO, 1995; WHO, 2000; WHO Expert Consultation, 2004)

There is a well-established association between a high BMI and an increased risk for morbidity rates of specific diseases and conditions. Some examples include coronary heart disease (CHD), T2DM, atherosclerosis, stroke, the MetS, hypertension and dyslipidaemia (Campbell, 2014; Morris, 2011; Phillips *et al*., 2013). The prevalence of the MetS is high among all BMI categories, but much higher in the obese category (Phillips *et al*., 2013). In recent years, several cancer types have also been associated with a high BMI, with an increased risk of post-menopausal breast cancer, colorectal, renal, endometrial, ovarian, and cervical cancer (Campbell, 2014; Kabat *et al*., 2015).

The use of BMI as an assessment of body composition does have its advantages. Weight and height are routine measurements in various fields of study. It is quick, easy to obtain and can be used in adults and children of both genders (Eston & Reilly, 2009; Phillips *et al*., 2013). However, BMI is a not the best and most accurate predictor of adiposity, as it is only an estimate of adiposity (Eston & Reilly, 2009). The use of BMI has also been heavily criticized by the fact that it does not describe or distinguish between lean and FM, as well as fat distribution (Phillips *et al*., 2013). In addition, BMI does not take into account the actual shape of the body itself (Eston & Reilly, 2009). Body shape (android and gynoid) has been shown to play a bigger role in disease states (Orbetzova *et al*., 2012; Ronco *et al*., 2008), and this will be described in a later section of this thesis.

1.4.2.3 Waist circumference, hip circumference & waist-hip-ratio

Waist circumference has been shown to be a good alternative measurement for assessing abdominal obesity as it accounts for BMI's limitations and correlates well with the gold standard methods (CT and MRI) (Stewart & Sutton, 2012). Currently WC is the only adiposity measurement used in the NCEP-ATP III and IDF MetS definitions (Expert Panel on Detection, Evaluation, and Treatment of

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High Blood Cholesterol in Adults, 2001; IDF, 2006; Phillips *et al*., 2013), while BMI is used in the WHO definition (Alberti & Zimmet, 1998; Phillips *et al*., 2013).

Waist circumference has been used to complement BMI for estimating obesity risk (Cornier *et al*., 2011), and studies have actually shown it to be a better predictor of mortality and disease risk than BMI (Brenner *et al*., 2010; Campbell, 2014; Kabat *et al*., 2015), as well as the incidence of various cancers (breast, endometrium and colorectal) (Kabat *et al*., 2015). Although WC is a simple, affordable and non-invasive measurement of body composition (Eston & Reilly, 2009), it has not been well-implemented in clinical settings as there are controversies regarding the specific area for accurate measurement of WC (Cornier *et al*., 2011). The use of WC as a measurement of abdominal obesity is also limited by the fact that it is sensitive to body size, as well as fat percentage and distribution (Krakauer & Krakauer, 2012).

Waist- and HC are used in combination to calculate WHR (Norton & Olds, 1996), which is an alternative index reflecting the accumulation of abdominal fat (Stewart & Sutton, 2012). The rationale behind this index is that the greater the accumulation of abdominal fat, the greater WC is relative to HC (Olds *et al*., 2013). It has been criticised for its use in assessment of body composition, as there is still no consensus on cut-off points defining obesity and central obesity (Cornier *et al*., 2011).

A WC of > 94 cm in men, and > 80 cm in women, and a WHR of 0.94 in men and 0.85 in women have been associated with an accumulation of visceral fat, which is associated with an increased risk for CVD and T2DM (Eston & Reilly, 2009; IDF, 2006; Stewart & Sutton, 2012). Waist circumference, HC and WHR have been shown to be more useful than BMI in predicting CVD, CHD and other metabolic-related disorders (Cornier *et al*., 2011; Kabat *et al*., 2015). In addition, HC and WHR have been suggested as an alternative index of body shape to predict mortality compared to BMI (Stewart & Sutton, 2012).

It should be noted that it is still uncertain which adiposity index is more strongly associated with the risk of cancer development (Krakauer & Krakauer, 2012). This could be due to differences in study designs and a lack of proper studies evaluating different anthropometric indexes in relation to cancer risk (Kabat *et al*., 2015). In addition, there is very little evidence in the literature on the association between body shape and predisposition to diseases including the MetS and cancer (Baltadjiev & Vladeva, 2014). A detailed discussion on body shapes will be the topic of the next section.

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1.4.3 Somatotyping: Body shape

1.4.3.1 Introduction

Somatotyping describes body shape and body composition (Eston & Reilly, 2009; Stewart & Sutton, 2012), and can be divided into anthropometric somatotyping and photoscopic somatotyping (Norton & Olds, 1996). Anthropometric somatotyping can be calculated using a combination of different anthropometric measurements (weight, height, skinfolds, bi-epicondylar breadths, and circumferences) (Norton & Olds, 1996). The gold standard for somatotyping is the combination of anthropometric and photoscopic evaluation (Eston & Reilly, 2009; Norton & Olds, 1996). Somatotyping has its advantages above routine anthropometrical measurements such as BMI and WC, as it indicates the difference between individuals with the same level of adiposity and BMI (Eston & Reilly, 2009; Norton & Olds, 1996). However, somatotyping is based on careful observation of the different components, and a trained eye is needed to perform this observation objectively (Eston & Reilly, 2009; Norton & Olds, 1996).

1.4.3.2 Components of photoscopic somatotyping

Photoscopic somatotyping, also known as anthroposcopic somatotyping, works on a rating system that is based on visual inspection of an individual itself or a photograph of the individual (Norton & Olds, 1996).

Somatotyping is a quantitative expression or description of an individual's morphological conformation (Eston & Reilly, 2009; Norton & Olds, 1996), and gives an overall summary of an individual's present physique as a whole entity (Ronco *et al*., 2008). Somatotyping gives a useful summary of a variety of measurements, as well as observations of the human body, which should only be interpreted as a whole entity (Eston & Reilly, 2009; Ronco *et al*., 2008).

Each component of somatotyping defines a specific aspect or trait of how the human body is built (Norton & Olds, 1996). This is expressed as a numeral on a continuous scale, which starts at zero and has no upper limit (Eston & Reilly, 2009; Norton & Olds, 1996).

The first component of somatotyping is known as endomorph describes the degree of adiposity of the body (Figure 1.9A) (Eston & Reilly, 2009; Norton & Olds, 1996). Physical aspects include degree of roundness of the body, softness of contours, and relative volume of the abdominal area (Norton & Olds, 1996).

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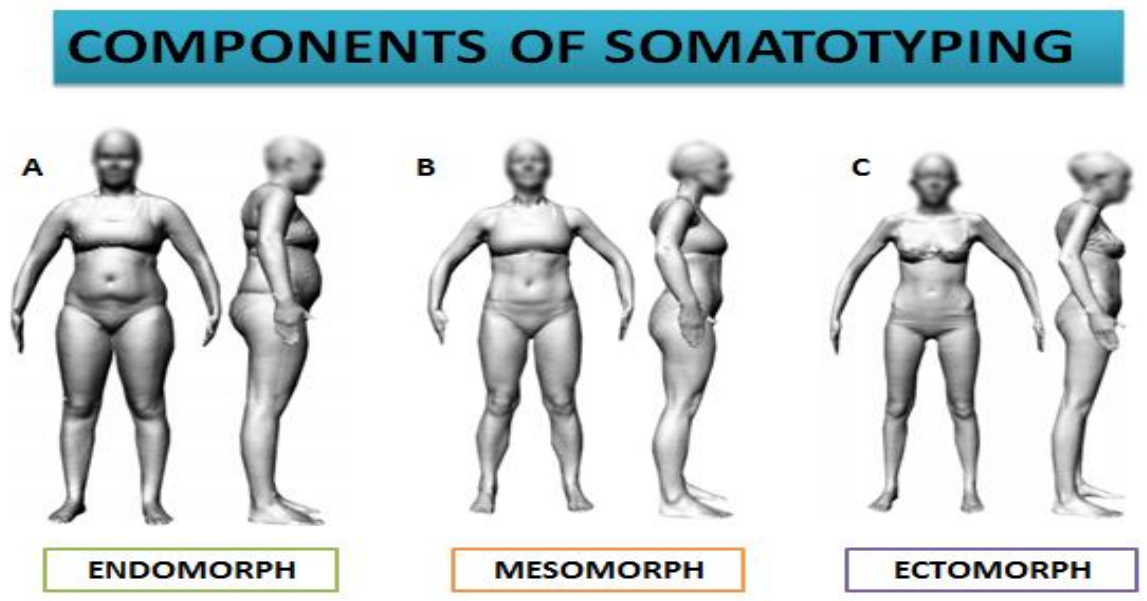

Figure 1.9: The different components of somatotyping.
(Adopted from: Olds *et al*., 2013)

The second component of somatotyping is known as mesomorph which describes the degree of musculoskeletal development of the body (Figure 1.9B) (Eston & Reilly, 2009; Norton & Olds, 1996). These physical aspects include robustness of the body in terms of muscle or bone, relative volume of the thoracic trunk, as well as muscle bulk (Norton & Olds, 1996).

The third component of somatotyping known as ectomorph, describes the degree of slenderness of the body (Figure 1.9C) (Eston & Reilly, 2009). Physical aspects include relative linearity of the body and the fragility of the limbs in the absence of any bulk muscle, fat or other tissue type (Eston & Reilly, 2009; Norton & Olds, 1996).

Other anthropometric methods have been proven to be more useful in describing body shape; however somatotyping requires minimal equipment and is a portable method (Norton & Olds, 1996). Somatotyping has been mostly used in fitness and athletic assessments (Eston & Reilly, 2009; Norton & Olds, 1996), however, its use within the medical field has been mainly related to obesity and CVD risk and not in cancer research (Ronco *et al*., 2008). This is attributed to the fact that more advanced techniques like three-dimensional whole body scanning are being used to determine the somatotype (Olds *et al*., 2013).

1.4.3.3 Android and gynoid body shape linked to disease states

There are three extreme somatotypes namely extreme endomorph, extreme mesomorph and extreme ectomorph (Norton & Olds, 1996). For the purposes of this thesis, only extreme endomorph which includes android and gynoid body shapes will be discussed, as it has been associated with CHD, CVD, Type 1 diabetes mellitus (T1DM), cardio-metabolic abnormalities, the MetS and cancer (Eston

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& Reilly, 2009; Momesso *et al*., 2011; Orbetzova *et al*., 2012; Ronco *et al*., 2008; Samsell *et al*., 2014).

It has been well-established that men and women have distinct fat distribution patterns (Stewart & Sutton, 2012). Men store adipose tissue predominantly in the abdominal region and women in the femoral-gluteal region (Patidar, 2013). This sex-specific distribution is thought to appear during puberty (Stewart & Sutton, 2012). However, due to changes in dietary patterns and an increase in prevalence of sedentary behavior, this sex-specific distribution is being changed, as more women present with an android body shape (Sardinha *et al*., 2012).

The android body shape is generally described to have the shape of an apple (Figure 1.10A). It is characterized by wide shoulders, arms, chest and a large abdomen, thus most of the fat being present in the abdominal region of the body (Patidar, 2013; Stewart & Sutton, 2012). With this shape, the gluteo-femoral region of the body, i.e. thighs, hips and legs are much leaner when compared to the abdominal region of the body (Patidar, 2013; Stewart & Sutton, 2012). The android body shape is predominately found in men and is a major risk factor for CVD, dyslipidaemia and IR (Eston & Reilly, 2009; Patidar, 2013). This is due to the fact that men store 20-30 % of their total body fat in the abdominal area, irrespective of obesity status (Donohoe *et al*., 2011).

In contrast, women only store a small quantity of total body fat in the abdominal area, and this quantity increases when a moderate level of obesity is reached (Donohoe *et al*., 2011). An increase in the prevalence of the android body shape is found in the MetS, overweight and/or obese females (Sardinha *et al*., 2012). In addition, the prevalence of an android body shape is also higher in post-menopausal female's *vs* pre-menopausal women (Noroozi *et al*., 2010).

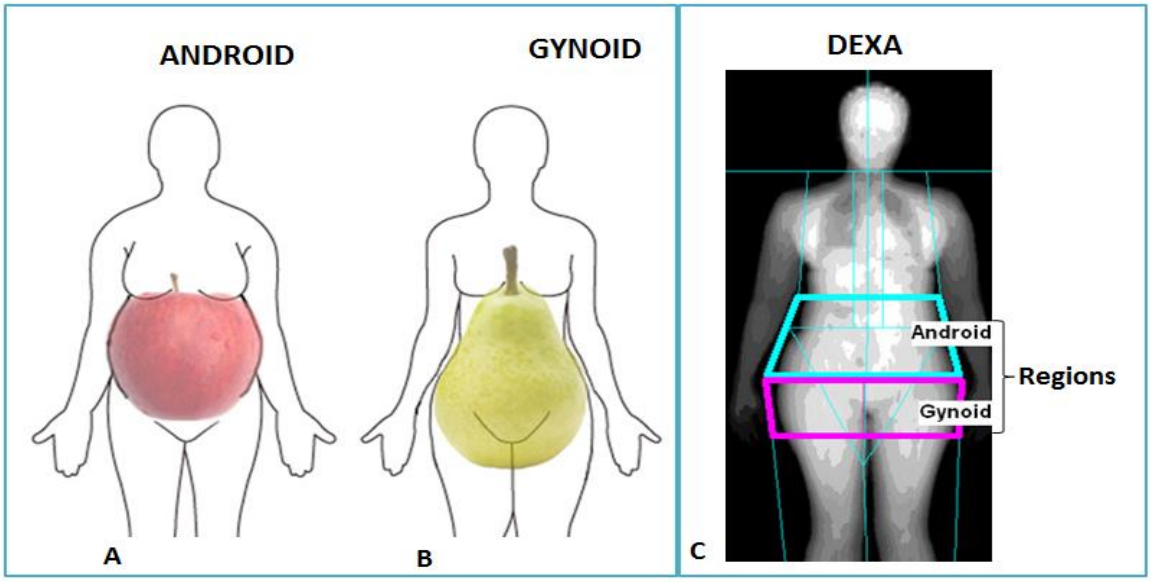

Figure 1.10: Android and gynoid body shapes.
(Adapted from: Dorgan *et al*., 2012)

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The gynoid body shape is generally described as pear-shaped (Figure 1.10B). It is characterized by broad thighs, hips and legs, with most of the storage fat being present in the gluteo-femoral region of the body (Patidar, 2013; Stewart & Sutton, 2012). The superior aspect (trunk) of the body is much leaner when compared to the gluteo-femoral region of the body (Patidar, 2013). This body shape is predominately found in women (Patidar, 2013; Ronco *et al*., 2008).

Gynoid obesity is thought to have cardio-protective properties (Stewart & Sutton, 2012). However, there is a lack of research focussing on hip-girths, despite the fact that this hip area is a major storage site for adipose tissue in women (Mastaglia *et al*., 2012). This is attributed to the fact that the most health-risk research has been focusing on abdominal obesity (Stewart & Sutton, 2012). Although a higher HC and WHR was found to be significantly associated with an increases risk for various diseases (Noroozi *et* al., 2010), previous findings suggest that a larger HC, to some extent, are protective in women (Stewart & Sutton, 2012). This may be related to the fact that the hip area is an alternative storage site for excess fat, with gynoid adipocytes possibly having an increased lipoprotein lipase activity and therefore a more effectively storage capability compared to visceral adipocytes (Stewart & Sutton, 2012; Wiklund *et al*., 2008).

Recent advances in software technologies have enabled the DEXA to assess regions of fat distribution (Figure 1.10C), therefore enabling the determination of an android-gynoid ratio (A/G ratio), which is related to disease risk, and has been found to be a good predictor of the MetS risk factors (Samsell *et al*., 2014). Since body shape is mostly gender-specific, it is thought that the gender-specific fat distribution pattern is sex hormone-regulated (Mastaglia *et al*., 2012). Oestrogen is a key regulator of adipose tissue distribution – after menopause oestrogen levels decline significantly and contributes to the distribution of adipose tissue to become more male-like and android shaped (Noroozi *et* al., 2010; Sardinha *et al*., 2012).

A study by Mastaglia *et al*. (2012) indicated that older, apparently healthy Caucasian women (60-69 years) had a significantly higher BMI compared to younger women (30-39 years) (23 ± 2 kg/m^2 *vs* 21 ± 2 kg/m^2, $p<0.04$). In addition, android fat mass (AFM) (1.5 ± 0.5 *vs* 1.3 ± 0.5 kg, $p<0.04$), and A/G ratio (0.7 *vs* 0.8, $p<0.01$) was significantly higher in post-menopausal women compared to pre-menopausal women (Mastaglia *et al*., 2012).

Wiklund *et al*. (2008) showed that AFM and gynoid fat mass (GFM) is associated with CVD risk in both men and women. In men, AFM was found to be the strongest predictor of most cardiovascular risk factors including TG, hypertension and impaired glucose tolerance (IGT), whereas in women, the A/G ratio was found to be the strongest predictor (Wiklund *et al*., 2008).

A case control study by Orbetzova *et al*. (2012) found that obese women displaying both android and gynoid body shapes had significantly higher FM and FFM *vs* age-matched controls. Mean leptin

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levels were found to be significantly higher in women with android obesity (21.28 ± 11.4 ng/ml units *vs* 10.02 ± 5.89 ng/ml, p<0.05) *vs* controls. In addition, android obesity had significantly higher mean insulin levels (20.13 ± 8.17 mIU/ml *vs* 10.47 ± 5.24 mIU/ml, p<0.05) compared to gynoid obesity (Orbetzova *et al*., 2012), suggesting, that an android fat distribution may play a role in the development of IR/hyperinsulinaemia.

Momesso *et al*. (2011) aimed to assess the relationship between body composition, MetS and IR in female patients with T1DM. Dual-energy X-ray absorptiometry revealed that women with T1DM and the MetS had a higher android fat distribution (41.9 ± 2.0 % *vs* 33.7 ± 1.8, p=0.004) and A/G ratio (0.9 ± 0.005 % *vs* 0.7 ± 0.03 %, p=0.0002) when compared to women with only T1DM. Total body fat, as well as gynoid fat percentage was not found to be significantly different between groups with and without the MetS. In addition, the estimated glucose disposal rate as an estimate of insulin sensitivity, was negatively associated with android fat percentage (r =-0.33, p=0.03), A/G ratio (r=-0.51, p=0.004), WC (r= -0.50, p< 0.001), as well as WHR (r=-0.63, p< 0.0001). Android fat percentage was also positively associated with LDL-c (r=0.38, p=0.01) and TG levels (r=0.35, p=0.019) and negatively correlated with HDL-c (r=-0.38, p=0.01). These results indicate that abdominal fat depositions are associated with the MetS and IR, which may lead to an increased risk for cardio-metabolic diseases (Momesso *et al*., 2011).

Namwongprom *et al*. (2014) found that height, weight and WC were significantly higher in participants with the MetS than those without the MetS. Regional fat distribution parameters, AFM, android lean mass, GFM, gynoid lean mass and A/G ratio, was significantly higher in those with the MetS compared to those without the syndrome (p<0.001 for all parameters). Significant positive correlations were also observed between TG, SBP, diastolic blood pressure (DBP), fasting plasma glucose (FPG) and several body composition parameters (p<0.05). To adjust for BMI, gender, birth weight and BMI, linear regression analysis revealed significant positive correlations with the MetS risk components score and WC (p<0.001), AFM (p<0.001) and A/G ratio (p<0.001). These results support evidence that abdominal fat is a major predictor of the MetS and other metabolic abnormalities (Namwongprom *et al*., 2014).

Body shape, increased weight, obesity and fat distribution patterns have been associated with an increased risk for cancer especially in women (Healy *et al*., 2010; Kim *et al*., 2007; Zhang *et al*., 2010[B]). However, these studies were based on anthropometric methods utilising BMI, WC or skin fold measurements to represent obesity or abdominal obesity. A study by Ronco *et al.* (2008) investigating whether body shape was associated with an increased risk for breast cancer development in women, found that of all the somatotype variables, only endomorph was significantly higher in cases *vs* controls (6.91 ± 1.96 vs 6.54 ± 1.81, p=0.004). In addition, a high endomorph somatotype was associated with an increased risk for breast cancer. When endomorph was distinguished by

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menopausal status, it was found that pre-menopausal women had an increased risk for breast cancer, whereas in post-menopausal women only the adjusted OR was significantly associated with breast cancer risk (OR 19.8, CI 95%, 1.00-3.90, p=0.03). Thus, endomorph was associated with an increased breast cancer risk irrespective of menopausal status (Ronco *et al.*, 2008). On the other hand, Dorgan et *al*. (2012) showed that total adiposity and body fat distribution were significantly inversely associated ($p<0.001$) with increased breast tissue density. In addition, A/G ratio was negatively correlated with denser breast tissue, which has also been linked to an increased risk of breast cancer (Dorgan *et al*., 2012).

Scientific evidence reveals a direct relationship between obesity, especially central obesity and chronic diseases including the MetS (Haghighatdoost *et al*., 2014). Thus, it is pivotal to make use of validated and reliable anthropometric indexes such as BMI, WC, WHR, to estimate disease risk associated with obesity and the MetS (Samsell *et al*., 2014).

1.4.4 A body shape index (ABSI)

The use of the BMI as a metric of obesity classification is limited by the fact that it does not have the ability to distinguish between fat and muscle mass, and only provide information on the distribution of fat mass (Haghighatdoost *et al*., 2014). Waist circumference has been recommended as measurement of abdominal obesity to account for BMI's limitations (Haghighatdoost *et al*., 2014). However, the use of WC as a measurement of abdominal obesity is limited by the fact that it is sensitive to body size, as well as TBF percentage and fat distribution (Kabat *et al*., 2015).

These limitations should be recognized; as evidence clearly shows that an increase in abdominal fat leads to an increased risk for the incidence and mortality of non-communicable diseases, while an increase in muscle mass decreases mortality risk (Haghighatdoost *et al*., 2014; Krakauer & Krakauer, 2012).

Due to the limitations and discrepancies for the use of BMI and WC, a new metric has been proposed, known as a body shape index (ABSI) (Krakauer & Krakauer, 2012). This new metric takes weight, height and WC into account, and is independent from BMI (Krakauer & Krakauer, 2012).

$$\boldsymbol{ABSI} = \frac{\boldsymbol{WC}}{(\boldsymbol{BMI})^{\frac{2}{3}}\ \boldsymbol{Height}^{\frac{1}{2}}}$$

The ABSI formula was derived from data obtained from the National Health and Nutrition Examination Survey (NHANES) (1999-2004), a survey based on mortality to better quantify the risk associated with abdominal obesity (Krakauer & Krakauer, 2012). Due to the fact that ABSI uses WC,

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height and weight, it may improve methods of assessing risks related to obesity and body composition that is not captured by BMI (Krakauer & Krakauer, 2014).

The ABSI is thought to be a more reliable method to measure body fat, because it takes height into account, as well as where fat is distributed (Krakauer & Krakauer, 2012). In addition, it is thought to give a better representation of overall body shape (Krakauer & Krakauer, 2012). A high ABSI value means that WC is higher than expected for a given height and weight, and this in turn corresponds to a larger abdominal area (Krakauer & Krakauer, 2012). A high ABSI value may also correspond to an increase in VAT compared to peripheral tissue (Krakauer & Krakauer, 2012). Furthermore, it may suggest that these individuals have decreased FFM and lean tissue, and it is for this reason that ABSI may be regarded as a better predictor for metabolic-related disease (Krakauer & Krakauer, 2014).

Advantages of ABSI include the fact that it accounts for a sub-linear increase of WC and BMI, and a non-linear association of WC with height (Krakauer & Krakauer, 2012; Krakauer & Krakauer, 2014). In addition, ABSI is a convenient method to use in a clinical setting as it only requires standard weight, height and WC measurements, which are routinely done (Krakauer & Krakauer, 2012; Krakauer & Krakauer, 2014).

To date, possible applications of ABSI include predicting all-cause mortality, CVD mortality, onset of T2DM, the MetS and high blood pressure to name but a few (Krakauer & Krakauer, 2014). However, data on ABSI and relative health risk are still controversial (Krakauer & Krakauer, 2012; Krakauer & Krakauer, 2014). Only one study has thus far shown that ABSI is a much stronger predictor of mortality than BMI and WC (Krakauer & Krakauer, 2012). This American-based study revealed that the risk factors for death increased by 1.13 % for each standard deviation increase in ABSI, and that the individuals in the highest ABSI quartile had death rates that were 61 % higher than those in the lowest quartile. In addition, the study showed that ABSI was significantly and positively correlated with abdominal adiposity ($r=0.45$, $p<0.05$) and negatively correlated with limb lean mass ($r=-0.26$, $p<0.05$). This study therefore concluded that a high ABSI value is a risk factor for pre-mature mortality in the general population. However, although ABSI predicted mortality risk across age, sex and weight, ethnic differences were evident, which limited the utility of ABSI. This further suggests that more studies are needed to strengthen the use of this metric (Krakauer & Krakauer, 2012).

Haghighatdoost *et al*. (2014) investigated the predictive ability of ABSI for the MetS and CVD risk compared to BMI and WHR in an Iranian population. In this study, a weak correlation between ABSI and numerous CVD risk factors (TC, TG, HDL-c, LDL-c, FPG, CRP, Apo lipoprotein A & B, SBP and DBP), as well as the MetS were evident. ABSI was found to be a weak predictor of both CVD risk and the MetS (Haghighatdoost *et al*., 2014), whereas in the case of T2DM and hypertension onset, ABSI showed a stronger predictive ability than BMI (Duncan *et al*., 2013; He & Chen, 2013).

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Biolo *et al.* (2015) assessed the efficiency of ABSI to predict variability of fat free mass index (FFMI) in men and women and reported that FFMI was negatively associated with ABSI and positively associated with BMI. Linear regression analysis showed that BMI and ABSI are both independent predictors of FFMI in men and women respectively. In addition, men and women with a lower ABSI showed a significantly greater FFMI, and the ABSI also positively correlated with log CRP ($r= 0.30$, $p< 0.05$) and negatively with a reciprocal of plasma insulin ($r= -0.25$, $p< 0.05$), which is an index of insulin sensitivity.

Evidence from various studies postulates the possibility that ABSI may be a useful index to possibly assess the association of obesity to the potential risk of developing certain cancer types. A study by Kabat *et al*. (2015) in post-menopausal women, investigated ABSI and its association with the incidence of specific cancer types. Firstly, ABSI was weakly associated with height ($r=0.047$), weight ($r=0.018$, and BMI ($r=-0.003$), however it was strongly associated with WHR ($r=0.75$, $p<0.0001$). The study further revealed that ABSI was not associated with an increased risk for breast and endometrial cancer, while BMI and WC were strong predictors. In addition, WC and WHR showed a stronger association with colorectal and kidney cancer when compared to BMI. While ABSI was a significant predictor for colorectal cancer (Harding *et al.*, 2015; Kabat *et al.*, 2015), WC showed a stronger association when compared to ABSI (Kabat *et al*., 2015). This study showed that ABSI does not pose any improvement for assessing the association between abdominal obesity and the risk of obesity-related cancers, since the association of WC and WHR with these cancers was much stronger compared to ABSI (Kabat *et al*., 2015)

Overall, evidence for the use of ABSI as a predictive tool for disease risk still remains controversial. It is suggested that ABSI's poor performance in estimating disease risk can be attributed to the fact that it has only been used to estimate total mortality and certain disease states. Further studies are therefore needed to investigate ABSI's potential association with cancer incidence, mortality and risk. In addition, ABSI has not been validated in other epidemiological studies which may differ in study population, outcomes, design, disease, and sample size.

1.5 Problem statement & relevance of the study

The MetS has been extensively studied in various populations with evidence pointing to a high prevalence of the MetS in all regions of the world (Awosan *et al.*, 2013; Hu *et al.*, 2004; Prasad *et al*., 2012; Tachang *et al.*, 2012). This is especially true for women as the prevalence of the MetS is higher in women *vs* men (Bhanushali *et al*., 2013). More specifically, there is an increase in the prevalence of abdominal obesity (android body shape) observed amongst women, which in turn, exacerbates the global prevalence of the MetS and its components amongst women (Momesso *et al*., 2011). Despite

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this, there are still limited data on the prevalence of the MetS and its components in South Africa, especially the Western Cape (Peer *et al*., 2015[A]).

In addition, evidence points to a link between the MetS, and its individual's components, inflammatory markers as well as the sex hormone profile, and the risk of developing various lifestyle cancers (Alokail *et al*., 2013; Roberts *et al*., 2010). It is still unclear what the specific role of the MetS or its individual components is in the pathophysiology of cancer development, or if the risk is only associated with the MetS as a whole entity (Sinicrope & Dannenberg, 2011). It is thus important to establish whether the MetS and its components are precursors for various lifestyle cancers since the MetS is reversible and preventable by lifestyle interventions (Redig & Munshi, 2010). Despite the clinical significance of the MetS and the risk of cancer development, the pathophysiology is still understudied in the South African setting.

South Africans need to recognize the burden and costliness of chronic lifestyle diseases. Early and accurate detection, diagnosis, treatment and prevention of the MetS and its components may decrease the disease burden of chronic diseases of lifestyle on private, as well as government healthcare providers in South Africa. In light of this, determining the prevalence of the MetS in the Western Cape will enable us to understand and resolve the effects of the MetS and its components to the risk of developing lifestyle cancers. This will provide significant insights into the extent of the MetS in the Western Cape, and in turn will contribute to the identification of underlying reasons.

In addition, determining and understanding the prevalence and disease risk associated with the MetS will provide information that will benefit public health systems, by implementing earlier therapeutic and preventative targets (Beltrán-Sánchez *et al*., 2013). There is also the possibility that the risk of cancer can be reduced by lifestyle interventions such as dietary changes, i.e. calorie restriction and increased physical activity (Agnoli *et al*., 2010). In addition, identifying and understanding the pathophysiology behind the MetS and the association with cancer will enable researchers to develop new therapeutic targets, as well as develop new diagnostic tests based on biological markers playing a role in this MetS-linked cancer development.

1.6 Aims and objectives

1.6.1 Aims

The primary aims were to describe the prevalence of the MetS and its association with cancer risk in a selected female population. Furthermore, we aimed to classify the female sample population based on their body shape, (android or gynoid), as well as body composition assessment measurements and describe biochemical associations that might increase their overall risk of cancer development.

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1.6.2 Objectives

1. Determine the prevalence of the MetS in a female population.
2. Determine if metabolic status and body-shape measurement/classification could predict changes in body composition, physiological and blood parameters and cancer risk.
3. To investigate possible correlations/associations between selected anthropometric, BIA and blood markers (IGF-1, CRP, E2 and SHBG), the MetS and cancer risk.

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CHAPTER 2: MATERIALS AND METHODS

2.1 Introduction

This chapter will focus on the materials and methods used for quantitative data collection. Other methods such as standard enzyme-linked immunosorbent assay's (ELISA) will be presented as a summary and the full method listed as an addendum.

2.2 Ethical considerations

This study received ethical approval from Stellenbosch University's Health Research Ethics Committee I (HREC I) (protocol number N13/04/052) (Appendix I). All principal investigators and researchers were familiarised with the ethical code of conduct, and were also certified in good clinical practice (GCP).

Farm workers from three different locations, which included Villiera Wines at the Owethu Clinic (Stellenbosch), Neethlingshof (Stellenbosch), and Solms-Delta Wine Estate (Franschhoek), were invited to attend an information session regarding the specific research project. Farm workers could then volunteer to participate in the study, where after 20-30 minutes visitations were scheduled for each volunteer on different days during the week.

At each scheduled visitation the researcher verbally explained to the participant what was expected of them during the data collection process, and that there would be a time investment of approximately 20-30 minutes. Participants were given time to thoroughly read through the participant information leaflet and consent form (Appendix II), after which they were free to ask questions if they had any uncertainties. After reading through the informed consent form, the document was signed. It was made very clear that all participants volunteered to take part in the study and could withdraw their consent at any point in time during data collection. The participants were also assured of their anonymity as no names were used on any data collection forms - only the principle investigator had access to personal information. All information was number coded and stored in a lockable cupboard and office. These number codes were used in all data capturing procedures, as well as laboratory analyses. For privacy reasons, all participants were spaced apart (with dividers) so that they could not hear or see each other. Following laboratory analyses, individual feedback session were provided to all volunteers at the respective data collection sites.

2.3 Study design and setting

The initial study design was intended to be a cohort (>6 years), with the focus on the effect of urbanization on health change or disease risk, on different strata i.e. deep rural, rural, peri-urban

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(includes farm workers) and urban. However, due to the collapsing of funds this current study's design is cross-sectional, with the focus on farm workers.

A cross-sectional, baseline descriptive study design was followed in the Stellenbosch Municipal region focussing on the surrounding wine estates in the Western Cape Province. Data collection commenced between March 2015 until July 2015, from three different locations; Owethu Clinic (Stellenbosch) on the Villiera Wine Estate, Neethlingshof Wine Estate (Stellenbosch), and Solms-Delta Wine Estate (Franschhoek).

2.4 Study population

The study population included volunteering women between the ages of 20-60 years.

2.5 Study inclusion and exclusion criteria

For eligibility into the study, the participants had to meet the following inclusion criteria: (1) they must be between the ages of 20-60 years, (2) must be permanent residents of the Western Cape Province (Winelands region), and (3) must have the ability to provide informed consent. Those who were ineligible were either younger or older than 20-60 years, not usual residents from the Western Cape Province, or pregnant or lactating at the time of data collection.

2.6 Selection of participants

The total number of successfully recruited, volunteering participants (which included both men and women) was n=191 (Figure 2.1). From this initial sample, n=42 men were excluded for this particular study, and n=2 women withdrew consent due to time constraints. Following data collection, a further n=7 women were excluded due to the inability to classify their body shape. Due to technical errors, n=12 women also had to be excluded. Thus, a total of n=63 participants were excluded.

The remaining n=128 women were then either classified as having the MetS or not, based on the IDF's criteria. This definition includes WC as the first compulsory criteria, based on population and country-specific cut-off values, and also recognizes that inflammation plays a role in the pathophysiology of the MetS (IDF, 2006).

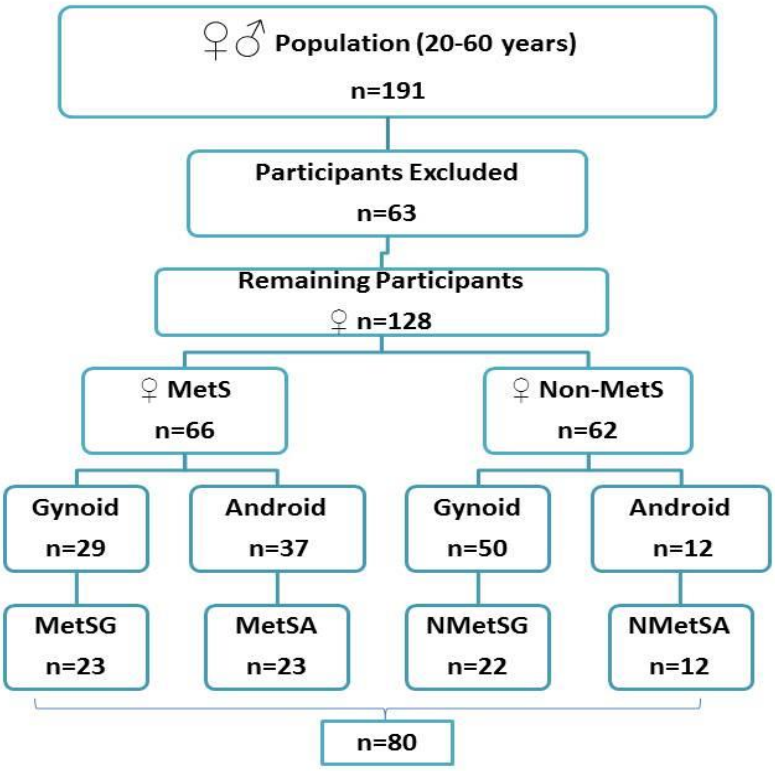

Figure 2.1: Selection process of participants for this current study.

The classification of participants was according to the IDF's guidelines, the following criteria were used. Central obesity, WC ≥ 80 cm for women, had to be present, plus any two of the following components: blood pressure (SBP ≥ 130 mmHg, and DBP ≥ 85 mmHg), fasting blood glucose (FBG) (≥ 5.6 mmol/L), HDL-c (< 1.3 mmol/L for women) or TG (≥ 1.7 mmol/L) (IDF, 2006). Following this classification, n=62 participants were divided into the non-MetS group, whereas n=66 were classified as having the MetS.

Female participants within the MetS or Non-MetS groups were also further classified according to body shape, to render the following groups (Figure 2.1):

1. MetS with gynoid body shape (MetSG) (n=29).
2. MetS with android body shape (MetSA) (n=37).
3. Non-MetS with gynoid body shape (NMetSG) (n=50).
4. Non-MetS with android body shape (NMetSA) (n=12).

In order to obtain a final sample size of n=80 for all data and laboratory analyses, the n=128 participants, belonging to the different groups, underwent random allocation to ensure an equal probability to be included in the final sample, with the exception of the NMetSA group, which only had n=12 participants (Figure 2.1).

The n=80 women were randomly allocated to the specific subgroups as follows (Figure 2.1):

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1. MetSG (n=23)
2. MetSA (n=23)
3. NMetSG (n=22)
4. NMetSA (n=12)

2.7 Data collection and handling

Each participant spent approximately 30 minutes completing a series of questionnaires, tests and assessments. All data files and questionnaires were number coded, and these number codes were used during all data capturing procedures, as well as laboratory analyses to ensure confidentiality and anonymity. Data collected from each participant was captured on separate data sheets, where after all the collected data were entered into an excel spread sheet and thoroughly checked for any missing values, inconsistencies or any typing errors. To further ensure anonymity, excel sheets were password protected.

2.8 Lifestyle questionnaires

2.8.1 Global physical activity questionnaire (GPAQ)

Physical activity was indirectly assessed using the global physical activity questionnaire (GPAQ) version 2 (WHO GPAQ, 2004) (Appendix III). This questionnaire is a standardized questionnaire developed by the WHO to assess physical activity in epidemiological field studies. It is a reliable physical activity assessment tool, and has been validated in several countries (Bull *et al*., 2009). The GPAQ consists of 16 questions, which were developed to assess various components of physical activity, i.e. intensity, duration and frequency. In addition, it assessed physical activity in three different settings i.e. occupational-related, transport-related and physical activity during leisure time (WHO GPAQ, 2004).

2.8.2 Smoking and drinking questionnaire

Smoking and drinking (alcohol) was indirectly assessed using a standardised questionnaire. Each questionnaire included questions regarding history of smoking/drinking, the quantity in relation to smoking/drinking, as well as how long participants have been smoking/drinking (Appendix IV).

2.9 Anthropometrical assessments

All anthropometrical measurements performed and included in this study were done according to guidelines set out by the International Society for the Advancement of Kinanthropometry (ISAK) (Olds *et al*., 2006; Stewart *et al*., 2011). Indices of body fat distribution included in this study were BMI, WC, HC, WHR, photoscopic somatotyping (Appendix V) and BIA (Appendix VI).

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In order to obtain information on body shape and body composition, all participants were informed during the consent process that they would have to remove some (not all) clothing for body dimension assessment. Any metal objects were also removed prior to the BIA assessment, to adhere to the standard pre-test BIA protocol. All measurements were done in a private, separate section of the examination room with a divider. All anthropometric measurements were measured in duplicate to ensure accuracy.

2.9.1 Base measurements: body mass and stretched stature

For base measurements, all study participants were instructed to remove all heavy clothing, shoes and any other additional clothing accessories (i.e. hats, head bands) to ensure measurement accuracy.

2.9.1.1 Body mass

Participants were requested to void before any anthropometrical assessment commenced. Before body mass was taken, the electronic scale was set to zero. The participants were instructed to stand in the centre of the scale (Seca 634; Seca United Kingdom, Birmingham, England) without any support, and with the weight evenly distributed between both feet. The weight measurement was taken to the nearest 0.01 kg.

2.9.1.2 Stretched stature

Each participant's height was measured using a portable, standard stadiometer (Leicester™; Leicester, England) without shoes. Participants were instructed to stand on the foot base of the stadiometer assuming the correct anatomical stance. Participants were then told to stand in an upright position (heels together) with the scapula and buttocks as close to the vertical sliding bar as possible, with arms relaxed. The participants' head was placed in the Frankfort plane, and using the sliding head board (Broca plane) of the stadiometer, the height was measured to the nearest 0.1 cm. The BMI (kg/m^2) was calculated using these base measurements.

2.9.2 Waist and hip circumference measurements

Waist circumference and HC measurements were assessed using a Lufkin tape measure (Lufkin, USA). The cross-hand technique was used for all circumference measurements. Participants were instructed to stand in an upright position with the abdomen relaxed, arms at sides and feet together. For the WC, the measuring tape was placed around the participant's waist between the 10th lower costal rib and the superior iliac crest at the narrowest plane of the abdomen (Eston & Reilly, 2009; Olds *et al*., 2006). For the HC, the measuring tape was placed around the largest area of the buttocks (i.e. around the gluteal muscles), with the participants' feet together. Both the WC and HC measurements were taken to the nearest 0.1 cm (Eston & Reilly, 2009; Olds *et al*., 2006). Waist-to-hip ratio was calculated by dividing WC by HC.

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2.9.3 Photoscopic somatotyping

Somatotyping was done using the photoscopic somatotyping technique (Norton & Olds, 1996). This technique involves the visual inspection of participants in order to evaluate the relative degree, presence or absence of each component of somatotype, in order to classify them as either android or gynoid body shape. The android body shape is generally described to have the shape of an apple, with most of the fat being present in the abdominal region (Patidar, 2013; Stewart & Sutton, 2012). The gynoid body shape is generally described to have the shape of a pear, with most of the fat being present in the gluteo-femoral region of the body, while the abdominal part of the body is much leaner (Patidar, 2013; Stewart & Sutton, 2012).

2.9.4 Bio-electrical impedance analysis (BIA)

Bio-electrical impedance analysis measurements were done using the multi-frequency Bioscan 920 II analyser (Maltron 920, UK), to assess FM (%), FFM (%) and muscle mass (kg). All participants were instructed to remove all forms of metal, which included jewellery, cell phones, money or any other electronic equipment that might interfere with the Bioscan 920 II multi frequency analyser during analysis A pre-test protocol was followed prior to measurements (Appendix VI).

2.9.4.1 Full body analysis

A full body analysis was conducted with the Maltron Bioscan 920 II analyser (Appendix VI). For the full body analysis, the participants were requested to lie on the examination bed in the supine position with their legs and arms slightly apart. The dominant (mostly right side) of the participants were always used for the BIA assessments. In some cases scar tissue, or prosthesis implants made accurate measurements difficult, in which the left side was used. The specific skin areas (hand and foot) used for testing were cleansed with 70 % v/v alcohol swabs (WebCol, USA, Illinois) before placement of the electrodes (Bodystat, United Kingdom). Prior to the measurement of a specific subject, their base measurements, i.e. weight, height, age, gender and ethnicity were entered in the Maltron Bioscan 920 II analyser.

The impedance of biological tissues was measured by placing the electrodes (with different channels and colours as seen in Figure 2.2) on the right hand and foot of the participant. Two current-introducing electrodes were placed at the dorsal surfaces of the hand (near metacarpal phalangeal joint) (Channel 1) and foot (near metatarsophalangeal joint) (Channel 3), while two voltage-detection electrodes were placed proximal of the wrist (pisiform prominence) (Channel 2), and ankle (medial and lateral malleoli) (Channel 4) respectively (Walter-Kroker *et al*., 2011). The Maltron Bioscan 920 II analyser applies various excitation currents i.e. 5, 50, 100 and 200 kHz *via* channel 1 and channel 3 and detects the voltage drops *via* channel 2 and channel 4.

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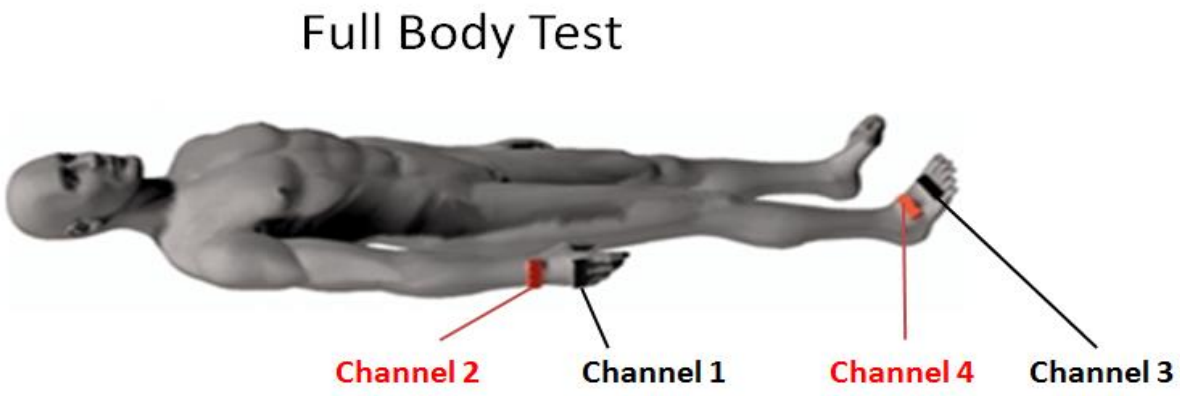

Figure 2.2: The correct electrode placement for the BIA full test.
(Adopted with permission from Maltron products Bioscan 920 II)

Participant' test results were initially stored on the Maltron Bioscan 920 II multi frequency analyser, and were later exported to Microsoft Office Excel 2010. All test results were number coded, and password protected to ensure anonymity.

2.10 Blood pressure

Blood pressure measurements were taken from each participant, and included in the IDF's criteria for the MetS classification. Participants were allowed to sit in a relaxed position for approximately ten minutes before any blood pressure measurements were taken. Blood pressure was measured on the right arm, using a calibrated aneroid sphygmomanometer (Erka Perfect Aneroid 48, Germany), and stethoscope (Littmann 3M stethoscope, USA) with an appropriate sized cuff.

Systolic blood pressure was recorded as the first Korotkoff sound, and DBP as the last Korotkoff sound (Frese *et al*., 2011). All blood pressure measurements were measured in duplicate to ensure accuracy. In cases where severe elevated blood pressure was detected participants were immediately referred to the clinic or health care providers.

2.11 Blood sampling and analysis

A Health Professions Council of South Africa (HPCSA) registered medical biological scientist (MW 0010782) and phlebotomist performed blood collection from the cubital vein of the right arm by aseptic techniques into different blood collection tubes.

One sodium fluoride (SF) tube (4 ml) was placed on ice and transported to the local PathCare laboratory in Stellenbosch for fasting blood glucose analysis. Two separate serum separator tubes (SST, 2x4 ml) were allowed to coagulate for 15 minutes at room temperature, after which it was centrifuged at 4000 revolutions per minute (rpm) for ten minutes at four °C. One of these tubes was

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send to PathCare for a fasting serum lipogram profile and insulin analysis. The remaining SST's serum was aliquotted into different Eppendorf tubes and frozen on dry ice, where after the samples were transported to the Department of Physiological Sciences' Molecular Laboratory and stored at -80 ˚C, until subsequent biochemical analysis. Serum samples were used to analyse CRP, IGF-1, oestrogen and SHBG respectively. Serum oestrogen, as well as sex hormone binding globulin (SHBG) were assessed by the local PathCare (Stellenbosch), whereas, IGF-1 and CRP were assessed using ELISA kits.

2.11.1 C-reactive protein (CRP) ELISA

In comparison to other inflammatory markers, CRP has a large range of reliable high sensitivity assays available. C-reactive protein is stable in plasma and serum samples; and stable during long term storage at -20 °C and -80 °C. It is furthermore cost-effective, not influenced by diurnal variation and nutrition, available in various countries, and correlates well with other inflammatory biomarkers (Allin & Nordestgaard, 2011; Brenner *et al*., 2014; Guo *et al*., 2015; Ingle & Patel, 2011; Salazar *et al*., 2014; Shrivastava *et al*., 2015). Thus, CRP was considered a suitable biomarker to describe inflammation.

A highly sensitive CRP ELISA (CRP human simple set ELISA kit®, Abcam, UK) kit was used to quantify CRP protein concentration in serum (appendix VII). The CRP concentration was assessed based on a solid phase sandwich-based ELISA assay, where the wells are pre-coated with an anti-tag antibody to immobilize the antibody-analyte complex (capture antibody\analyte\detector antibody). The CRP top standard (2000 pg/ml) was reconstituted with 0.2 ml water before making serial dilutions for the standard curve with the following concentrations: 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.3 pg/ml and 15.6 pg/ml. Fifty µl of each of the diluted standards, including a blank were added to the first 8 wells (in duplicate), whereas the rest of the wells were filled with different samples and the antibody cocktail (capture and detector antibodies to all wells) and allowed to incubate for one hour at room temperature. After incubation, the fluid was aspirated and wash buffer was added to each assay well (250 µl/well), and this step was repeated three times. This was followed by the addition of the 3, 3′, 5, 5′-tetramethylbenzidine (TMB) substrate solution, with the reaction brought to a stop after ten minutes with the addition of the stop solution. The assays' sensitivity ranges from 15.63 pg/ml – 1000 pg/ml with a minimal detectable dose of 4 pg/ml (Appendix VII).

The optical densities of all participant samples were measured at 450nm on an EL800 universal microplate reader (Bio-tek Instruments, Weltevreden Park, South Africa) within 15 minutes after the reaction was stopped. The optical density was directly (linearly) proportional to the CRP protein concentration of the participant sample. The concentration of all the participant samples was calculated by the formula produced by the standard curve. Serum samples were diluted 25 000x after

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consideration of relevant literature (Alwachi *et al*., 2013; Barinas-Mitchell *et al*., 2001; den Engelsen *et al*., 2012; Lapice *et al*., 2009; Laugsand *et al*., 2011; Santos *et al*., 2005; Vidyasagar *et al*., 2013).

2.11.2 Insulin-like growth factor-1 (IGF-1) ELISA

A highly sensitive IGF-1 ELISA (IGF-1 human ELISA kit®, Abcam, UK) was used to quantify IGF-1 concentration in serum (appendix VIII). This assay is based on a similar principle as the CRP ELISA, the only exception being the assays' sensitivity range, from (0.1 ng/ml – 30 ng/ml), the minimal detectable dose of (< 0.2 ng/ml).

The optical densities of all participant samples were also measured at 450nm on an EL800 universal microplate reader (Bio-tek Instruments, Weltevreden Park, South Africa) within 15 minutes after the reaction was stopped. Serum samples were diluted 20x after consideration of relevant literature (Friedrich *et al*., 2012; Friedrich *et al*., 2013; Gram *et al*., 2006; Kaushal *et al*., 2004; Oh *et al*., 2012; Schernhammer *et al.*, 2007; Schmitz *et al*., 2011)

5.12 Statistical analysis

All collected data was captured in Microsoft Office Excel 2010. Statistica Software version 12 (StatSoft, Inc., USA) was used to analyse data and the level of significance set as $p<0.05$.

All results are reported as means ± standard error of the mean (SEM) or medians depending on normality of data. For normally distributed data, student t-tests were performed to determine the difference in variables between two groups, and factorial analysis of variance (ANOVA) with Bonferonni *post hoc* test was done to establish significance between the four respective groups.

For data that was not normally distributed, non-parametric tests were performed. Mann-Whitney U tests were employed to determine the difference in variables between two groups and a one-way Kruskal-Wallis ANOVA was performed to determine the difference in variables between all the study groups. Pearson correlations were also used on selected parameters in each group and 2D scatter plots were drawn up in excel.

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CHAPTER 3: RESULTS

3.1 Context of the chapter

This chapter focussed on the results obtained in the current cross-sectional observational study. The results are presented in a logical flow, interpretable format, according to the aims and objectives as described in Chapter one. The chapter will further describe the study population, followed by a thorough portrayal of the population according to metabolic status and body shape. Finally, the chapter will make use of correlations to determine possible associations between selected parameters of physiological interest.

3.2 Descriptive profile of the study population

From the total sample (n=80) women, n=46 were identified by using IDF criteria with the MetS (57.5 %), while n=34 did not present with the MetS (42.5 %) (Figure 3.1A). Considering body shape, n=23 women in the MetS group exhibited a gynoid body shape, and n=23 women had an android body shape (Figure 3.1B). Sixty five percent, and 35 % of the women in the non-MetS group presented with a gynoid (n=22) and android (n=12) body shape respectively (Figure 3.1C).

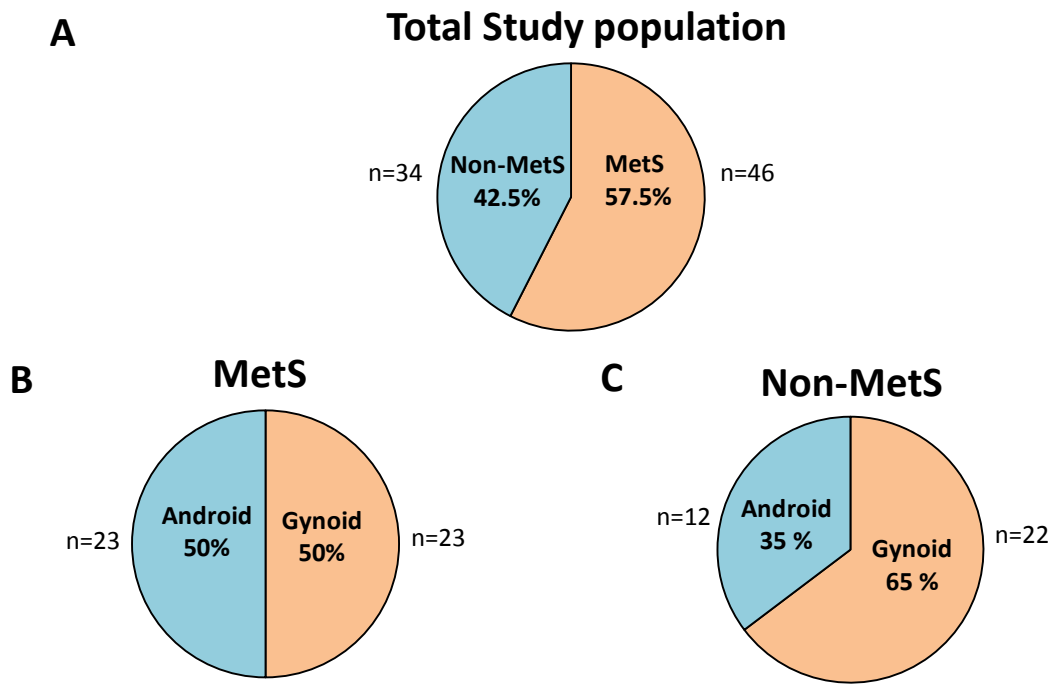

Figure 3.1: The distribution of women in (A) the total study population according to metabolic status, (B) the MetS, or (C) the Non-MetS groups according to body shape.

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3.3 Descriptive profile of the MetS and non-MetS groups

3.3.1 Anthropometric and BIA characteristics

The first aim of this study was to describe and compare women with and without the MetS in relation to age and body compositional differences. The women in the MetS group were significantly older compared to the non-MetS group (40.07 ± 1.58 *vs* 32.21 ± 1.36 years, $p<0.001$) (Table 3.1). Participants in the MetS group also displayed a significantly higher body mass (BM), BMI, WC, HC, WHR, and FM (%) than their non-MetS counterparts ($p<0.001$ for all except WHR: $p<0.05$), while FFM (%) was significantly lower ($p<0.001$). No significant differences were observed for height, ABSI, and muscle mass between these groups.

Table 3.1: Summary of anthropometric and BIA characteristics for the MetS and non-MetS groups.

Variable	MetS (n=46)	Non-MetS (n=34)	p-value
Age (years)	40.1 ± 1.6	32.2 ± 1.4	p<0.001
BM (kg)	84.81 ± 2.54	69.10 ± 3.43	p<0.001
Height (m)	1.57 ± 0.01	1.58 ± 0.01	ns
BMI (kg/m^2)	34.4 ± 0.9	27.4 ± 1.2	p<0.001
WC (cm)	93.10 ± 1.55	79.48 ± 2.28	p<0.001
HC (cm)	114.66 ± 1.96	102.64 ± 2.63	p<0.001
WHR	0.81 ± 0.01	0.78 ± 0.01	p<0.05
ABSI ($m^{11/6} kg^{-2/3}$)	0.07 ± 0.00	0.07 ± 0.00	ns
FM (%)	42.42 ± 1.64	32.04 ± 2.74	p<0.001
FFM (%)	57.58 ± 1.64	67.96 ± 2.74	p<0.001
Muscle mass (kg)	22.75 ± 1.32	19.87 ± 0.75	ns

All values are presented as mean ± SEM. Student t-tests were employed, and $p<0.05$ were considered as statistically significant. ns=not significant

BMI categories between women in the MetS and non-MetS groups

With particular reference to BMI, approximately two thirds of the MetS population were classified as being obese (67 %), 31 % as overweight, and two percent as having a normal weight (Figure 3.2A). In the non-MetS group, 47 % of the females were overweight, 29 % had a normal weight, 21 % were obese, and only three percent were underweight (Figure 3.2B).

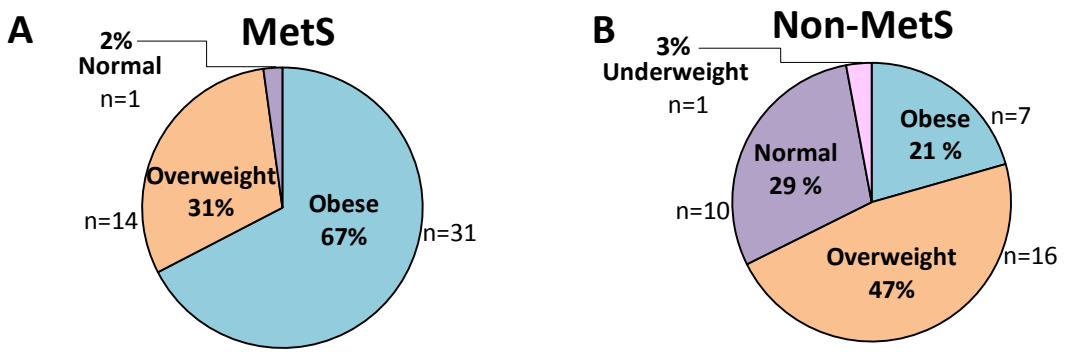

Figure 3.2: Distribution of women in (A) the MetS and (B) the non-MetS groups according to the respective BMI categories.

3.3.2 Physiological and biochemical blood parameters

Analysis of specific physiological and biochemical blood parameters revealed significantly higher SBP, DBP, FBG, fasting insulin, LDL-c, TG and FAI in the MetS women, while HDL-c and SHBG were significantly lower (Table 3.2). All other parameters measured were found to not be significantly different between the groups.

Table 3.2: Summary of physiological and biochemical blood parameters for the MetS and non-MetS groups

All values are presented as mean ± SEM with the exception of E2 and FAI which are presented as median ± IQR. Student t-

Variable	MetS (n=46)	Non-MetS (n=34)	p-value
SBP (mmHg)	148.8 ± 3.8	125.1 ± 3.0	p<0.001
DBP (mmHg	92.2 ± 2.2	77.4 ± 1.5	p<0.001
FBG (mmol/L)	6.22 ± 0.51	4.67 ± 0.12	p<0.05
Fasting Insulin (mIU/L)	35.69 ± 6.37	17.36 ± 2.07	p<0.05
TC (mmol/L)	4.85 ± 0.16	4.47 ± 0.20	ns
HDL-c (mmol/L)	1.02 ± 0.02	1.35 ± 0.06	p<0.01
LDL-c (mmol/L)	3.13 ± 0.14	2.64 ± 0.16	p<0.05
TG (mmol/L)	1.85 ± 0.16	1.02 ± 0.07	p<0.01
CRP (mg/L)*	10.86 ± 1.53	6.68 ± 1.29	ns
IGF-1 (ng/ml)#	79.50 ± 11.00	61.85 ± 12.35	ns
E2 (pmol/L)	160.50 ± 75-218	137.00 ± 75-307	ns
Female T (nmol/L)	1.2 ± 0.05	0.55 ± 0.08	ns
SHBG (nmol/L)	42.57 ± 3.65	73.84 ± 12.52	p<0.01
FAI	1.20 ± 0.80-7.75	0.70 ± 0.30-1.70	p<0.05

tests were employed for all parameters except for the E2 and FAI, where Mann-Whitney U tests were employed. p<0.05 was considered as statistically significant.*: Only n=64 samples included for analysis. #: Only n=61 samples included for analysis. ns=not significant

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3.3.3 Distribution and prevalence of the MetS risk factors

Apart from the fact that approximately 57.5 % of the study population presented with the MetS, we further aimed to describe the distribution of the MetS risk factors, as well as to establish which of the individual risk factors were more prevalent.

Figure 3.3A illustrated that 57 % of the women presenting with the MetS, had three of the MetS risk factors (n=26), 28 % had four risk factors (n=13), and 15 % presented with five risk factors (n=7). In the non-MetS group, 18 % of the women presented with no risk factors (n=6), 35 % had one risk factor (n=12), 44 % had two risk factors (n=15), and 3 % had three risk factors (n=1) with the exception of WC (Figure 3.3B).

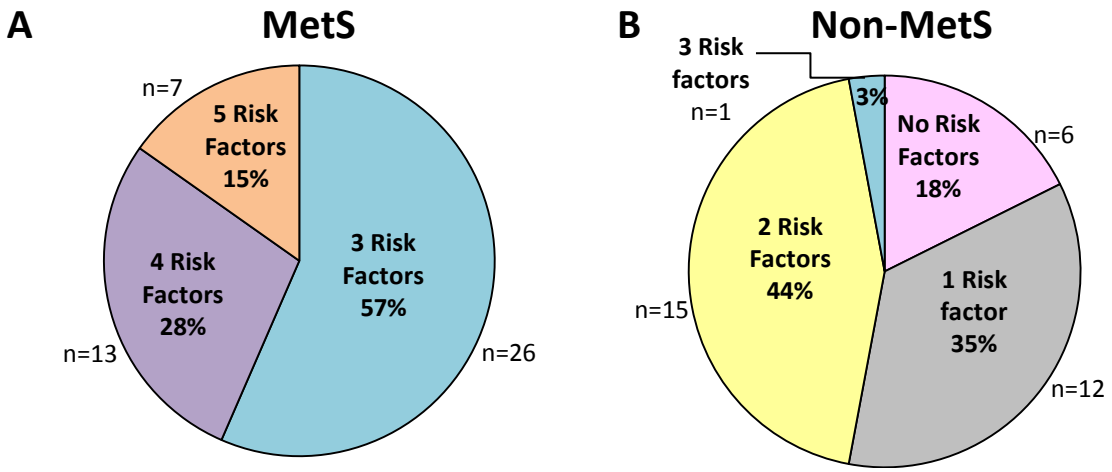


Figure 3.3: Distribution of women in (A) the MetS, and (B) non-MetS groups according to the number of MetS risk factors present.

For all the women in this particular study (irrespective of their metabolic status), the most prevalent risk factor was an increased WC (73.8 %, n=59) (Figure 3.4). This was closely followed by elevated blood pressure (BP), and decreased HDL-c levels (68.8 % for both, n=55), with approximately 27.5 % presenting with elevated TG levels (n=22), and 24.0 % with elevated FBG (n=19). When the metabolic status was considered, the most prominent risk factors still remained WC, BP and HDL-c for both the MetS and non-MetS groups (Figure 3.5).

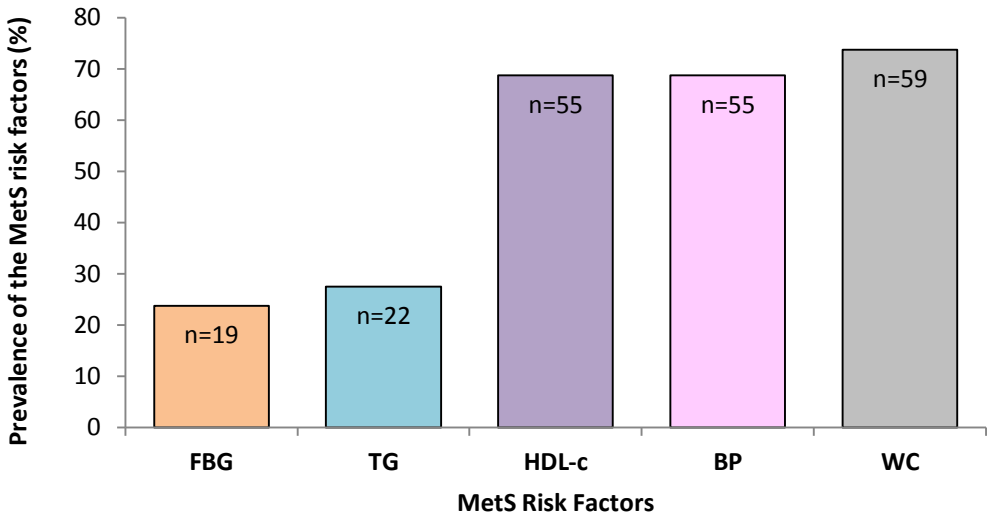


Figure 3.4 Prevalence of the MetS risk factors in women, irrespective of metabolic status.

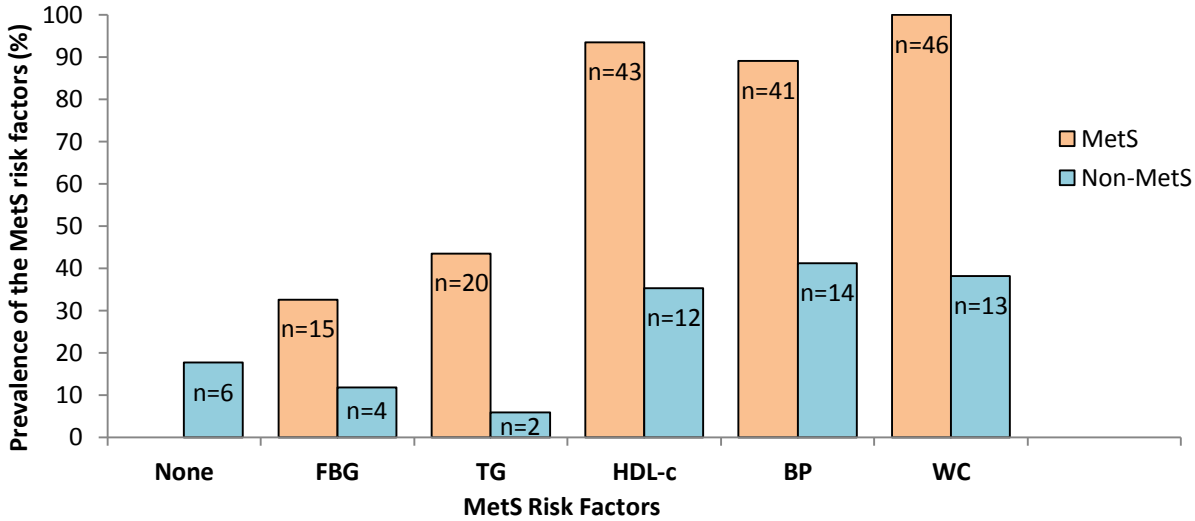


Figure 3.5: Prevalence of the MetS risk factors in women according to their metabolic status.

3.4 Descriptive profile of the gynoid and android body shape groups

3.4.1 Anthropometric and BIA characteristics

Our next aim was to determine whether body shape had an effect on body composition, irrespective of the metabolic status. The women in the android group were significantly older compared to the women from the gynoid group (40.09 ± 1.68 *vs* 34.11 ± 1.49 years, $p<0.01$) (Table 3.3). Women in the android group also displayed a significantly higher BM, BMI, WC, HC, WHR, ABSI and muscle mass (kg), compared to the gynoid body-shaped group. No significant differences were observed for height, FM (%) and FFM (%) in either groups.

Table 3.3: Summary of and BIA characteristics for the gynoid and android groups.

Variable	Gynoid (n=45)	Android (n=35)	p-value
Age (years)	34.1 ± 1.5	40.1 ± 1.7	p<0.01
BM (kg)	72.06 ± 2.60	85.94 ± 3.46	p<0.01
Height (m)	1.58 ± 0.01	1.57 ± 0.01	ns
BMI (kg/m^2)	28.8 ± 1.0	34.8 ± 1.2	p<0.001
WC (cm)	81.32 ± 1.76	95.02 ± 1.96	p<0.001
HC (cm)	106.23 ± 2.04	113.81 ± 2.78	p<0.05
WHR	0.77 ± 0.01	0.84 ± 0.01	p<0.001
ABSI ($m^{11/6} kg^{-2/3}$)	0.0696 ± 0.00	0.0718 ± 0.00	p<0.05
FM (%)	35.63 ± 2.01	41.08 ± 2.51	ns
FFM (%)	64.38 ± 2.01	58.93 ± 2.51	ns
Muscle mass (kg)	19.90 ± 0.44	23.61 ± 1.78	p<0.05

All values are presented as mean ± SEM. Student t-tests were employed, and p<0.05 were considered as statistically significant. ns=not significant

3.4.2 Physiological and biochemical blood parameters

No significant differences were observed in any of the physiological and biochemical blood parameters listed in Table 3.4.

Table 3.4: Summary of physiological and biochemical blood parameters for the gynoid and android body shape groups.

Variable	Gynoid (n=45)	Android (n=35)	p-value
SBP (mmHg)	135.8 ± 3.5	142.5 ± 4.6	ns
DBP (mmHg)	83.6 ± 2.0	88.9 ± 2.6	ns
FBG (mmol/L)	5.42 ± 0.41	5.75 ± 0.47	ns
Fasting Insulin (mIU/L)	26.94 ± 4.04	29.14 ± 7.27	ns
TC (mmol/L)	4.58 ± 0.16	4.82 ± 0.20	ns
HDL-c (mmol/L)	1.20 ± 0.05	1.11 ± 0.04	ns
LDL-c (mmol/L)	2.83 ± 0.14	3.04 ± 0.17	ns
TG (mmol/L)	1.35 ± 0.11	1.69 ± 0.19	ns
CRP (mg/L)*	8.06 ± 1.08	10.83 ± 2.02	ns
IGF-1 (ng/ml)[#]	83.54 ± 12.40	62.37 ± 10.95	ns
E2 (pmol/L)	137 ± 75-219	167 ± 91-280	ns
Female T (nmol/L)	0.58 ± 0.05	0.59 ± 0.08	ns
SHBG (nmol/L)	63.89 ± 9.82	45.53 ± 4.58	ns
FAI	1.2 ± 0.45-1.95	1.1 ± 0.40-2.10	ns

All values are presented as mean ± SEM with the exception of E2 and FAI which are presented as median ± IQR. Student t-tests were employed for all parameters except for the E2 and FAI, where Mann-Whitney U tests were employed. $p<0.05$ was considered as statistically significant. *: Only n=64 samples included for analysis. [#]: Only n=61 samples included for analysis. ns=not significant

3.5 Differences between respective groups according to both metabolic status and body shape

For a more thorough investigation into the effect of metabolic status and body shape, various anthropometric, BIA and blood-specific parameters were compared between the following four respective groups: (a) the MetS with gynoid body shape (MetSG) group, (b) the non-MetS with gynoid body shape (NMetSG) group, (c) the MetS with android body shape (MetSA) group, and (d) the non-MetS with android body shape (NMetSA) group.

3.5.1 Anthropometric measurements

3.5.1.1 Base measurements: body mass (BM) and height

The participants in the MetSG group (Figure 3.6A) showed significantly larger BM compared to the NMetSG group ($p<0.001$). Similar results were also observed between the MetSA and NMetSA group

(p<0.01) (Figure 3.6A). No significant differences were observed for height between any of the respective groups (Figure 3.6B).

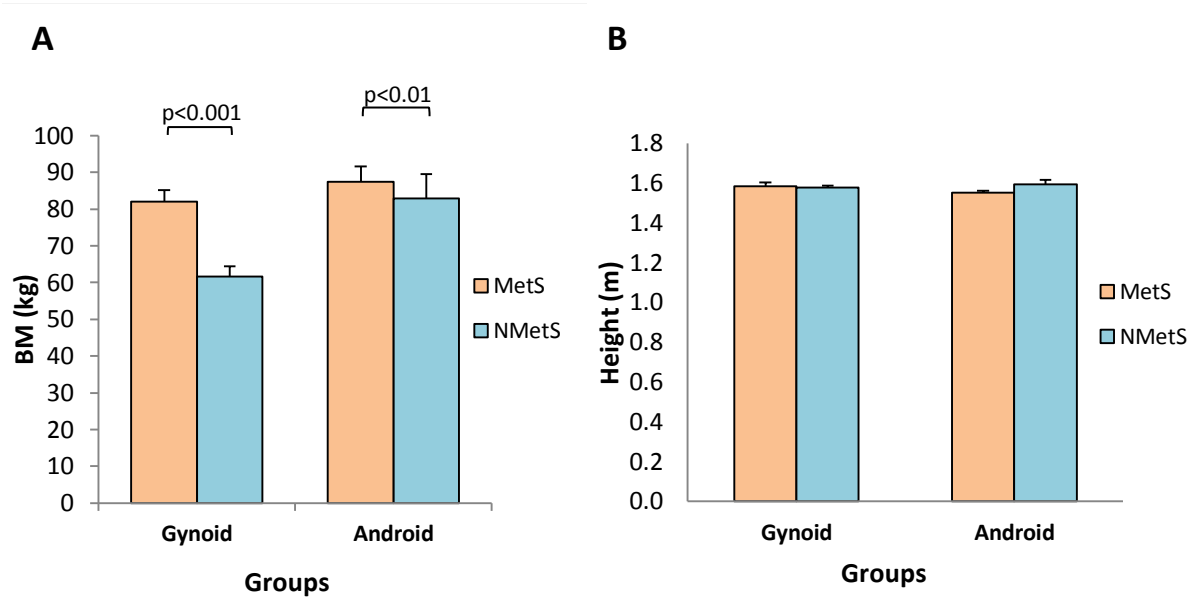


Figure 3.6: Base measurements, which include (A) BM and (B) height, between the respective groups.

3.5.1.2 Hip circumference (HC) and waist-hip-ratio (WHR)

The MetSG group's participants showed a significantly larger HC compared to the NMetSG group (p<0.01), however, this difference was not evident in the android group (Figure 3.7A). Although the WHR did not show any significant differences between the MetS and non-MetS groups for either body shape, the women in the NMetSA group showed a significantly higher WHR compared to those in the NMetSG group (p<0.001) (Figure 3.7B).

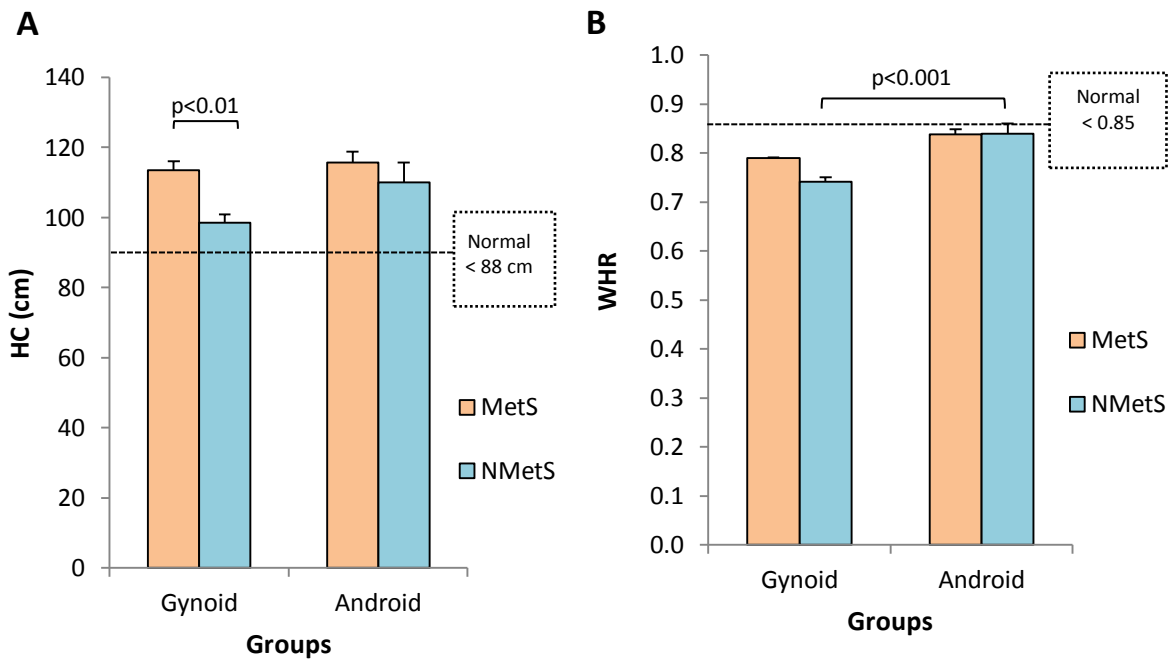


Figure 3.7: Hip circumference (A), and WHR (B) per body shape and metabolic syndrome status. The dotted lines represent normal levels for the respective parameters (WHO, 2008).

3.5.1.3 Body mass index (BMI) and a body shape index (ABSI)

Similar to the results observed for BM, significant differences in BMI were apparent between the MetSG, MetSA and their respective non-MetS body shape groups ($p<0.001$ for gynoid, and $p<0.01$ for android) (Figure 3.8A). No significant differences were observed for ABSI (Figure 3.8B).

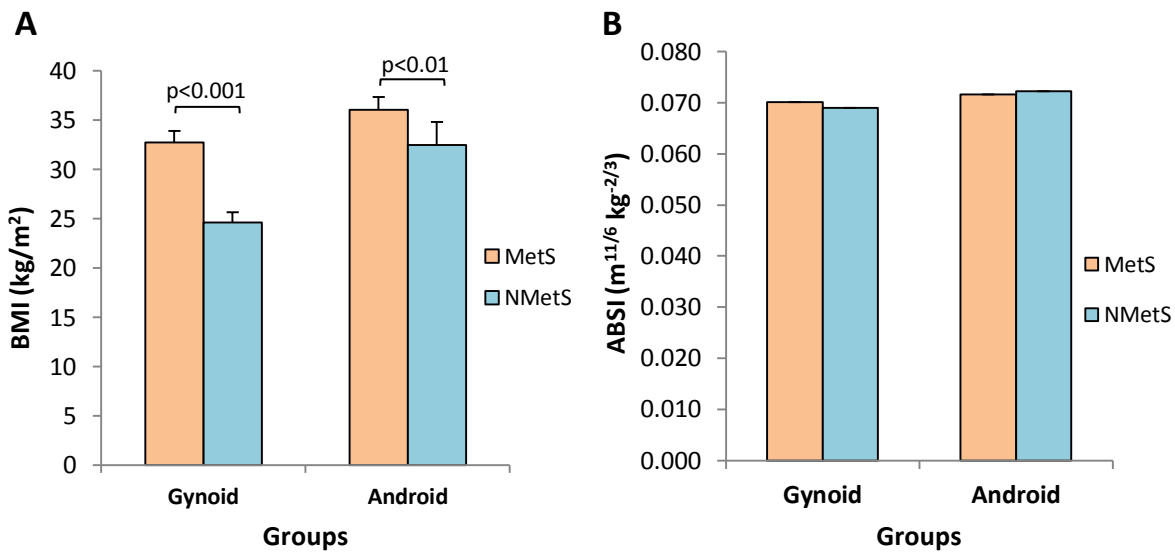


Figure 3.8: BMI (A), and ABSI (B) per body shape and metabolic syndrome status.

3.5.2 Bio-electrical impedance assessments

The participants in the MetSG group had a significantly higher FM (%) (Figure 3.9A) and significantly lower FFM (%) (Figure 3.9B) compared to the NMetSG group ($p<0.01$ for both). No differences were observed for muscle mass (Figure 3.9C).

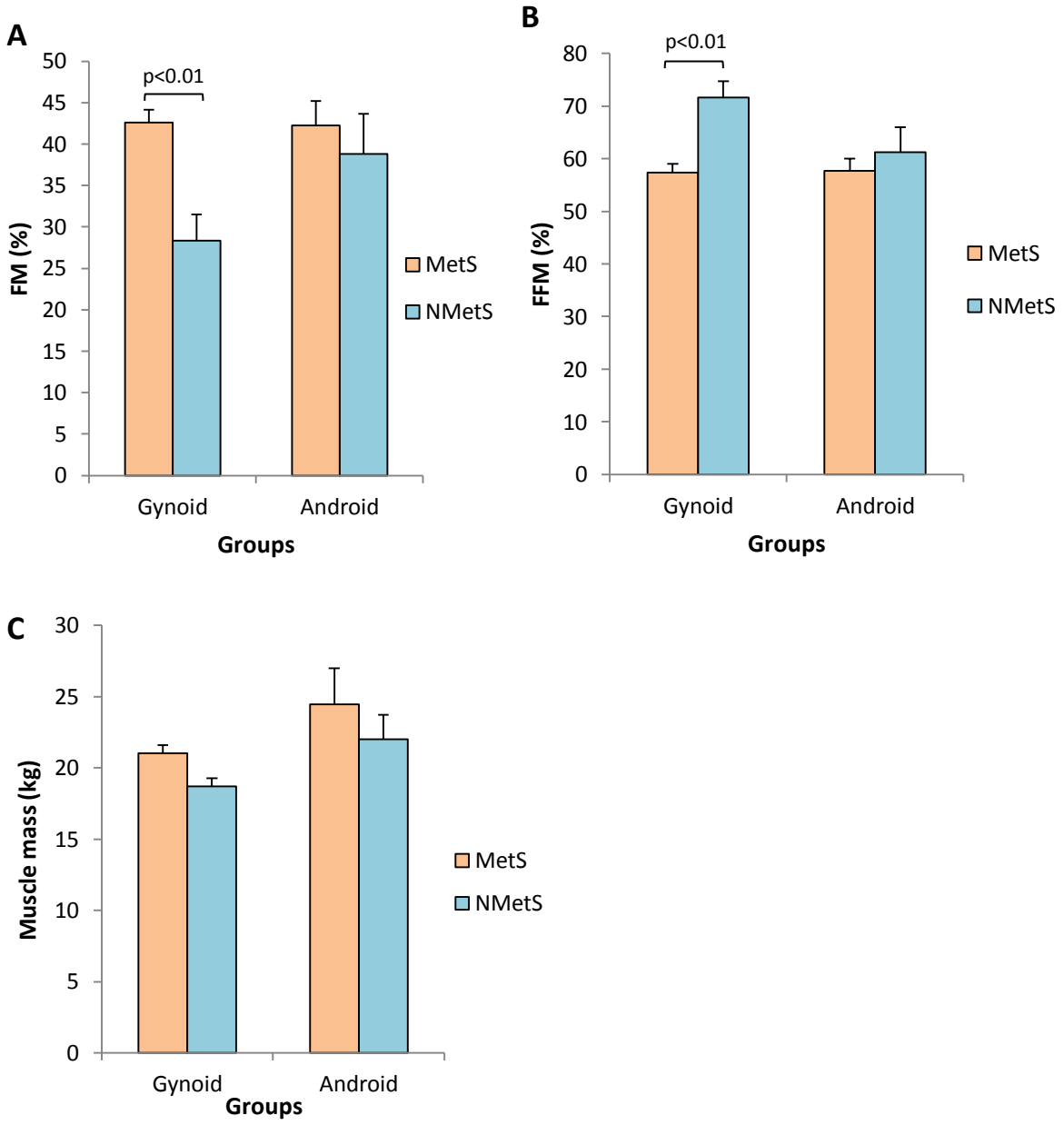


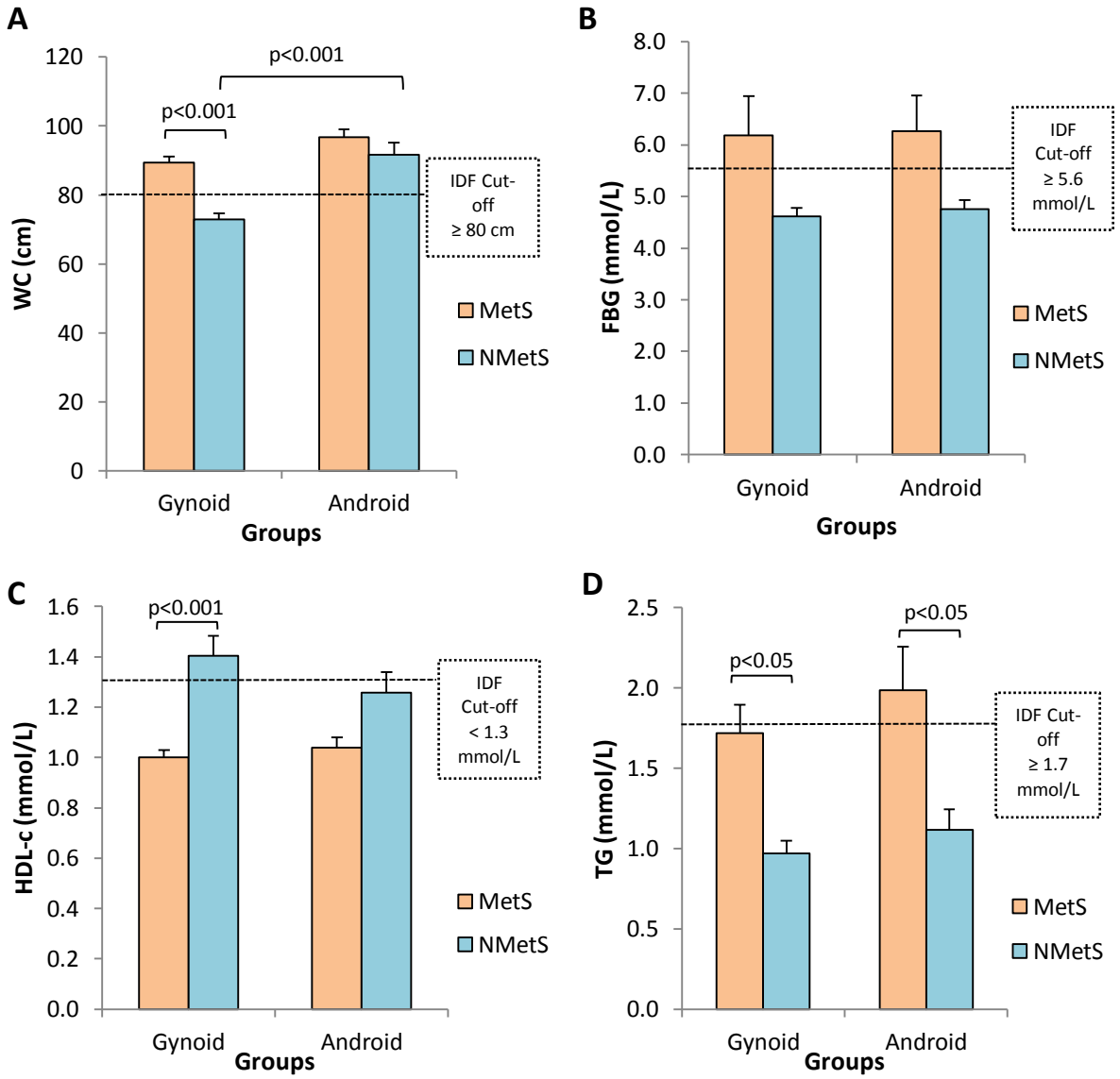
Figure 3.9: BIA measurements including (A) FM (%), (B) FFM (%), and (C) muscle mass (kg) for the respective groups.

3.5.3 The MetS risk factor measurements

Women in the MetSG group displayed a significantly larger WC compared to their NMetSG counterparts ($p<0.001$), however these results were not evident in the android groups. Overall, the WC for the NMetSA group was significantly larger than that of the NMetSG group ($p<0.001$) (Figure 3.10A). No significant differences were observed for FBG between any of the groups (Figure 3.10B).

The women in the MetSG group furthermore showed both a significantly lower HDL-c and elevated TG levels compared to the women in the NMetSG group ($p<0.001$ for HDL-c and $p<0.05$ for TG) (Figure 3.10C&D). No other significant differences were observed for HDL-c, but TG levels were also significantly lower in the NMetSA group *vs* the MetSA group ($p<0.05$).

Similar to that observed for HDL-c and TG, the non-MetS groups displayed significant lower SBP and DBP *vs* the MetS groups, irrespective of body shape (Figure 3.10E&F).



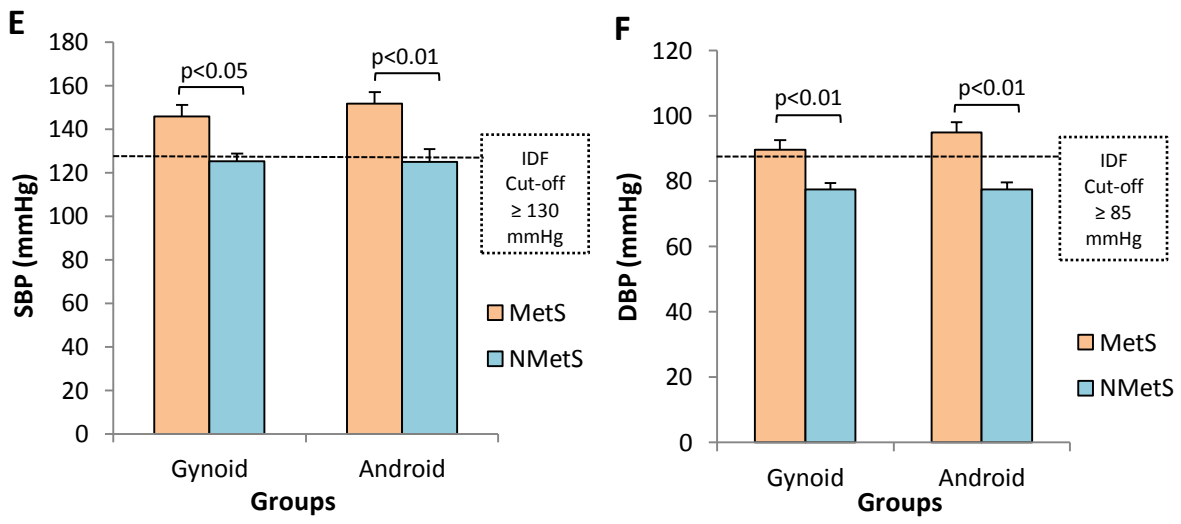


Figure 3.10: MetS risk factors according to IDF criteria for (A) WC, (B) FBG, (C) HDL-c, (D) TG, (E) SBP, and (F) DBP. The dotted lines represent IDF cut-off criteria (IDF, 2006).

3.5.4 Total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-c)

No significant differences were observed for both TC and LDL-c (Figure 3.11).

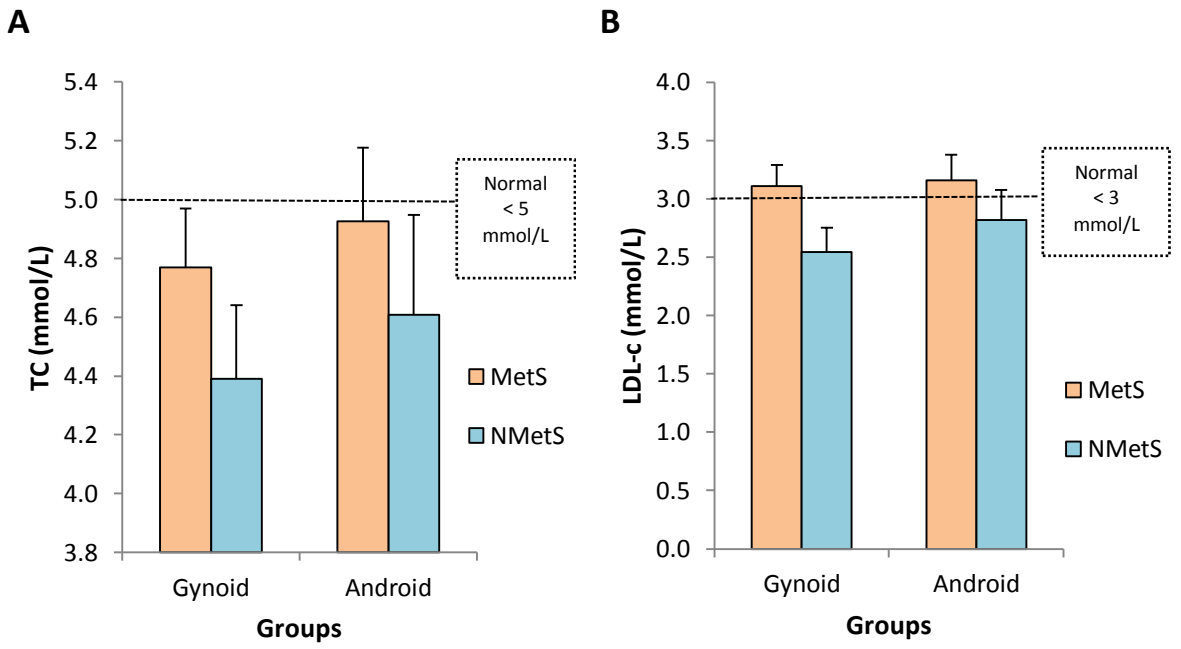


Figure 3.11: Blood analysis for (A) TC, and (B) LDL-c. The dotted lines represent normal cut-off criteria (Klug *et al.*, 2015).

3.5.5 Fasting insulin and insulin-like growth factor-1 (IGF-1)

No significant differences were observed for fasting insulin or IGF-1 between any of the groups (Figure 3.12).

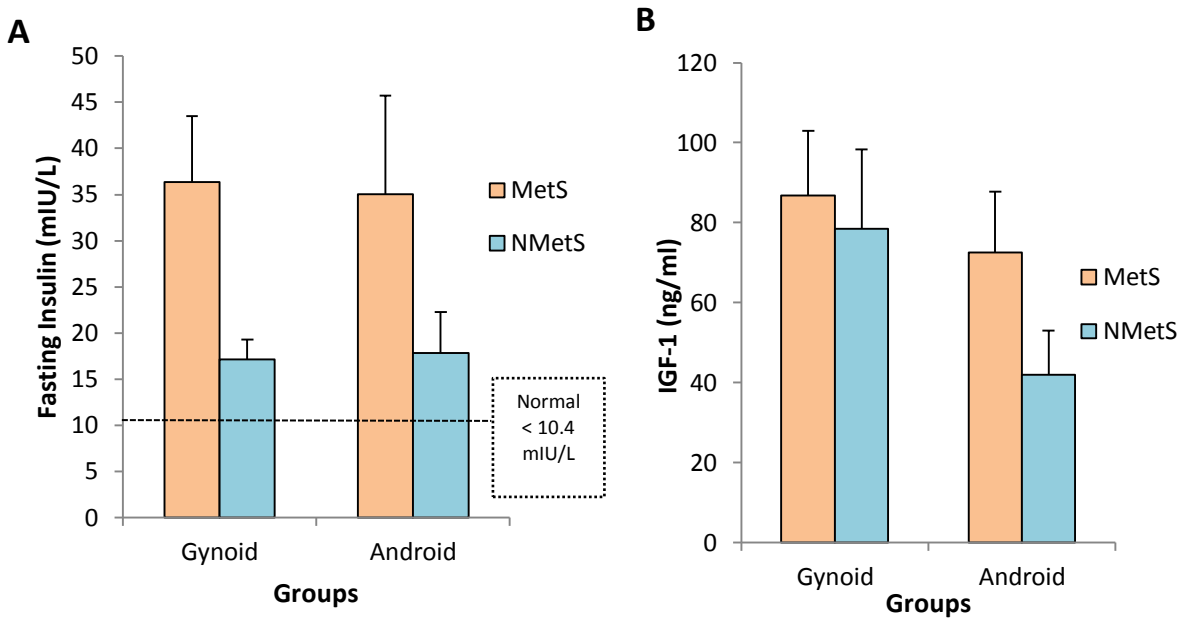


Figure 3.12: Fasting insulin (A), and IGF-1 (B) concentrations for the groups according to body shape and metabolic status. The dotted line represents normal cut-off criteria (reference range supplied by local pathcare laboratory, Stellenbosch).

3.5.6 C-reactive protein (CRP)

Similar to the data obtained for fasting insulin, IGF-1, TC and LDL-c, no significant differences were observed for CRP (Figure 3.13).

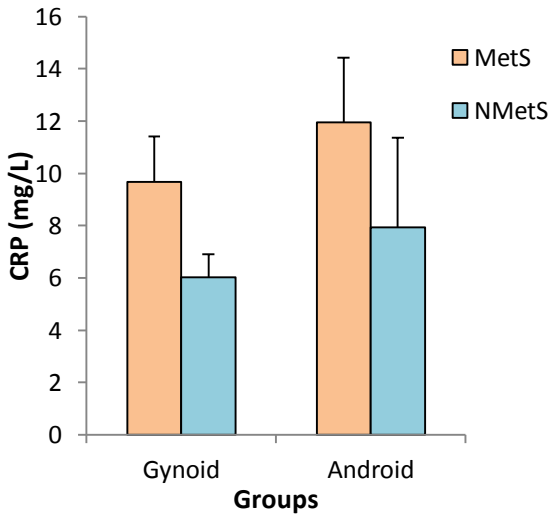


Figure 3.13: CRP concentrations for the different groups according to body shape and metabolic status.

3.5.7 Oestrogen (E2)

No significant differences for oestrogen concentration for the different groups were also reported (Figure 3.14).

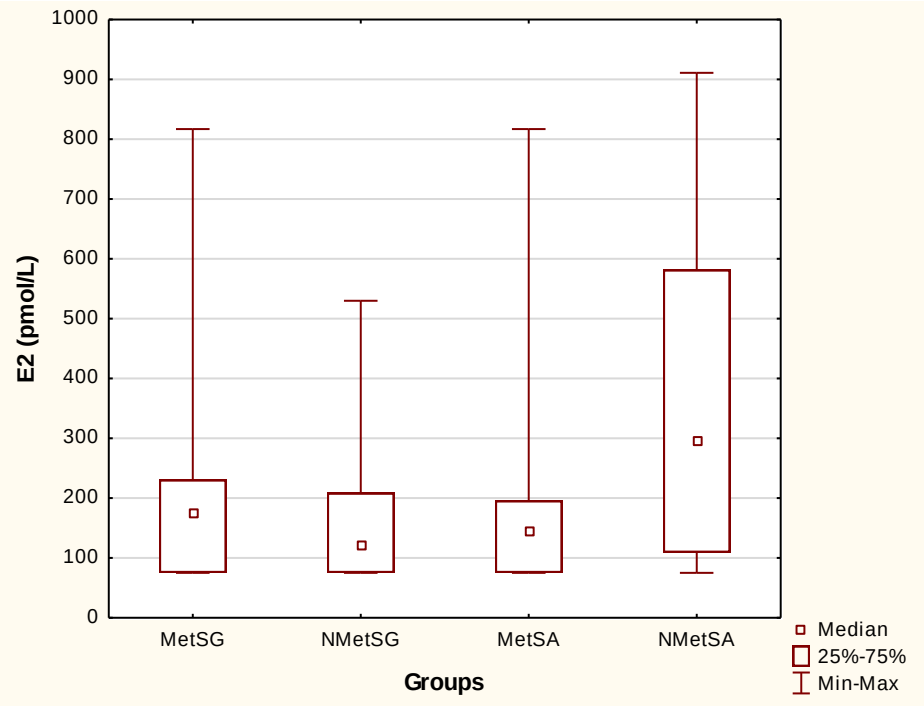


Figure 3.14: Median E2 concentrations with inter quartile ranges measured in the women belonging to the different groups.

3.5.8 Female testosterone (T), and sex hormone binding globulin (SHBG)

No significant differences were observed for female T, or SHBG in any of the different groups (Figure 3.15). From the normal level (Kozakowski & Zgliczyński, 2013), as indicated by the dotted line in Figure 3.15A for female T, females in all four groups displayed levels well within normal physiological range.

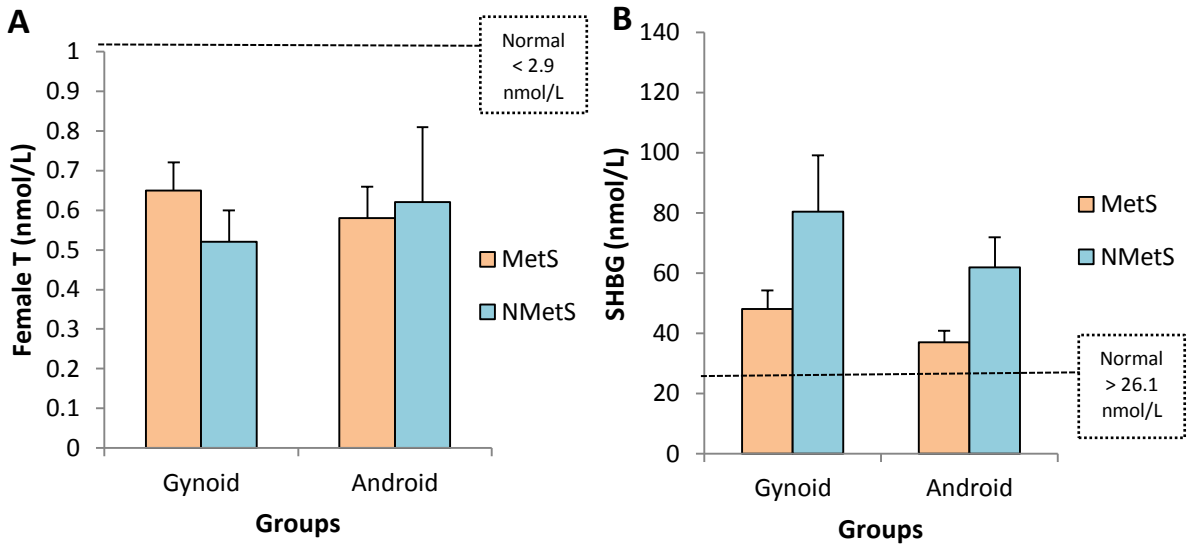


Figure 3.15: Female-specific hormone analyses for (A) female T and (B) SHBG for the different body shape and metabolic status groups. The dotted lines represent normal cut-off criteria (Kozakowski & Zgliczyński, 2013).

3.5.9 Free androgen index (FAI)

Comparable to E2, no significant differences for FAI were observed between any of the groups (Figure 3.16).

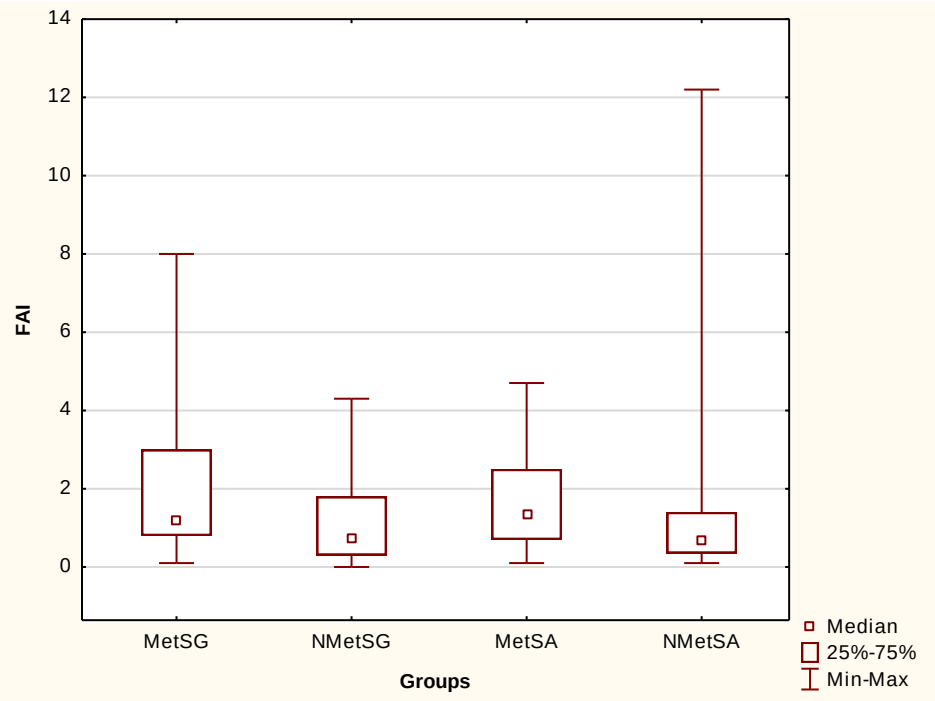


Figure 3.16: Median FAI between the different groups according to body shape and metabolic status as a measure of androgen excess.

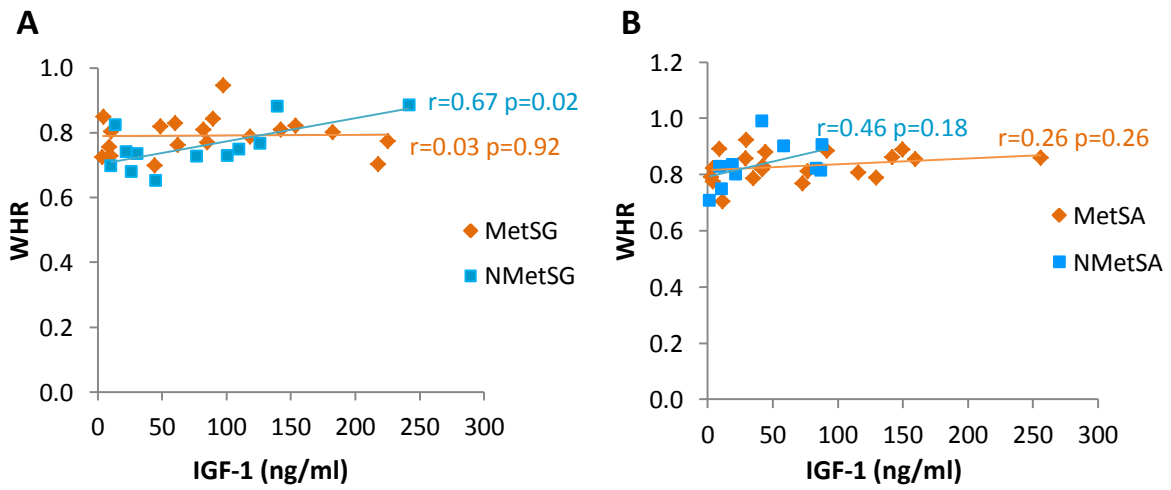
3.6 Correlation analysis

Pearson correlation analysis were performed on selected anthropometric and biochemical parameters to establish any significant physiological relationships. For all correlation purposes, the MetSG and NMetSG groups will first be graphically presented, followed by the MetSA and NMetSA groups. For correlation analyses, r-values greater than 0.70 will be regarded as very strong, r-values between 0.40 and 0.69 as strong, r-values between 0.30 and 0.39 as moderate and r-values between 0.20 and 0.29 as weak relationships. Any r-value less than 0.19 are indicative of no relationship (Scally, 2014).

3.6.1 Insulin-like growth factor-1 (IGF-1) and anthropometric parameters

Insulin-like growth factor-1 concentration positively correlated with the WHR for the women in all four study groups (Figure 3.17A&B). However, the correlations for both the MetS group's gynoid and android were insignificant. Strong correlations were evident in both non-MetS groups, but only the NMetSG group showed significance (r=0.67, p=0.02). When FM (%) was correlated with IGF-1 (Figure 3.17C&D), only the android groups showed a significant difference. The MetSA group displayed a strong positive correlation (r=0.45, p=0.05), whereas a very strong, negative correlation was found in the NMetSA group (r=-0.81, p=0.01).

Insulin-like growth factor-1 concentration was negatively correlated to FFM (%) (Figure 3.17E&F) for the MetSG, NMetSG and MetSA groups, but a very strong positive correlation was evident for the NMetSA group. No significance was noted in any of the gynoid groups; however both the android groups showed significance (r=-0.45, p=0.05 for the MetSA and r=0.81, p=0.01 for the NMetSA groups respectively). Another correlation that was associated with IGF-1 included fasting insulin. A significant strong negative correlation was shown between fasting insulin and FM (%) in the women from the MetSA group (r=-0.46, p=0.03) (Appendix IX).



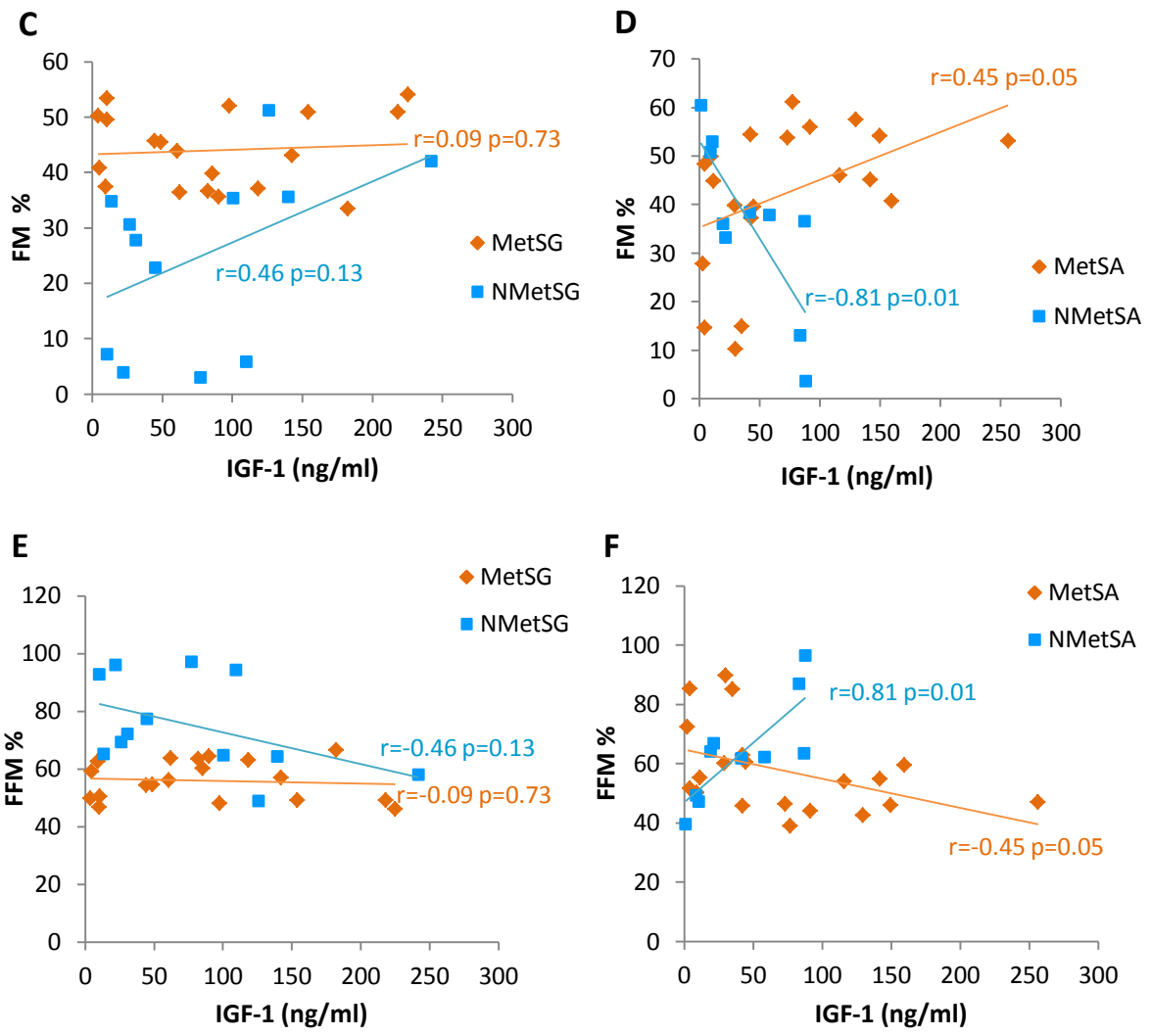


Figure 3.17: Correlations between IGF-1 and WHR, FM% and FFM% for the gynoid and android groups according to the metabolic status. Correlations for IGF-1 and WHR are displayed in (A&B), IGF-1 and FM % in (C&D), and IGF-1 and FFM % in (E&F).

3.6.2 C-reactive protein and anthropometric parameters

Positive correlations were evident in all the study groups when CRP was correlated with BMI (Figure 3.18A&B), with the exception of the NMetSG group which, showed a strong negative correlation. Only the MetSA groups' correlation was shown to be significant (r=0.60, p=0.00). When CRP was correlated with WC (Figure 3.18C&D), significance was obtained in three of the four groups. The NMetSG group revealed a significant, strong negative correlation (r=-0.58, p=0.02), whereas both the MetSG and MetSA groups showed significant strong positive correlations (r=0.47, p=0.04 for MetSG and r=0.49, p=0.03 for MetSA).

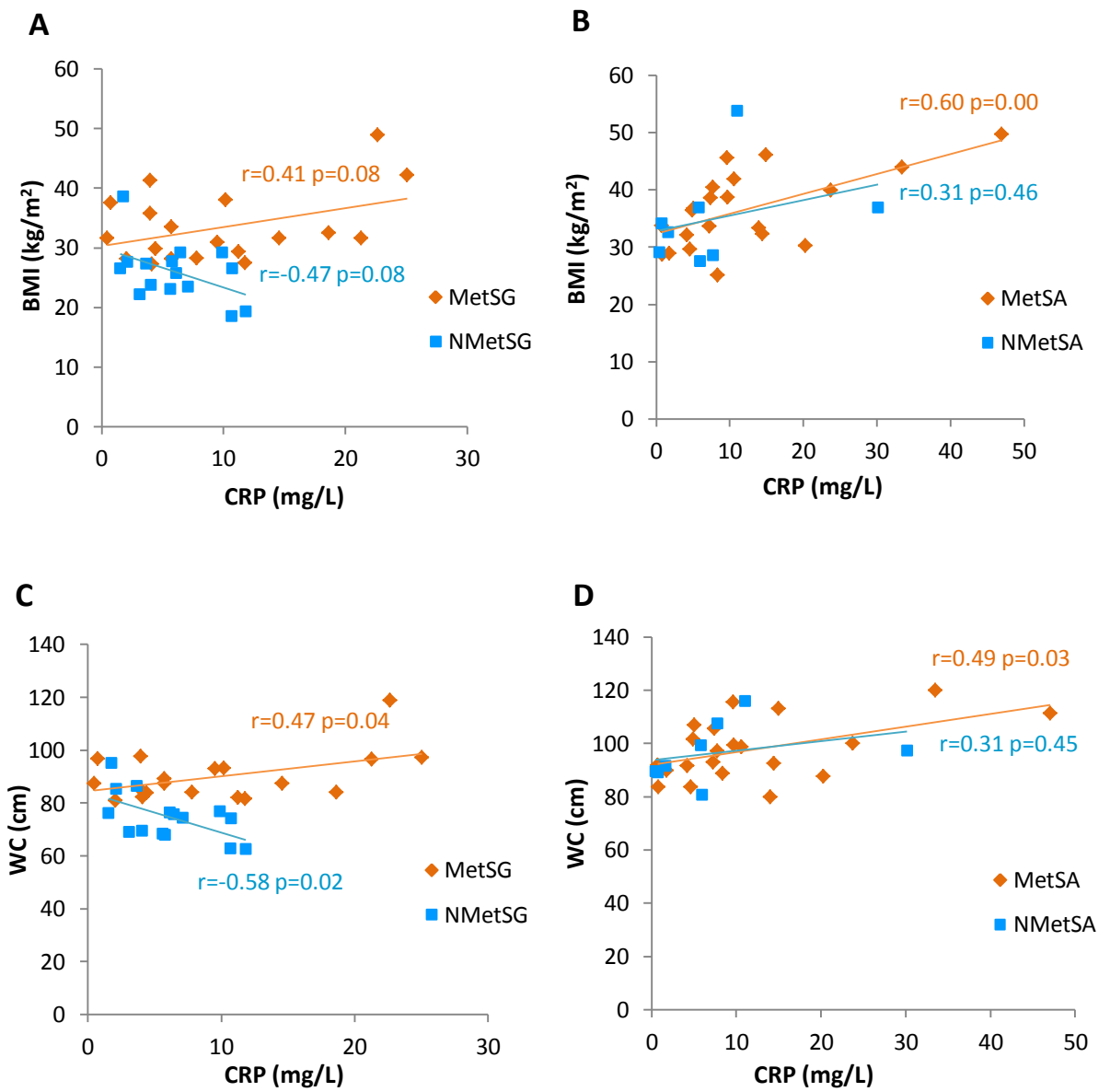


Figure 3.18: Correlations between MetS and non-MetS for the gynoid and android groups against CRP and BMI (A&B), and CRP and WC (C&D).

3.6.3 Sex hormone binding globulin, and selected parameters

Pearson correlation analysis further revealed a strong positive and significant correlation between E2 and BMI (r=0.52, p=0.01) (Figure 3.19A), E2 and fasting insulin (r=0.43, p=0.05) (Figure 3.19C), and a strong negative correlation between SHBG and fasting insulin (r=-0.52, p=0.04) (Figure 3.19E) in women from the MetSG group. Negative correlations were observed for all other groups (Figure 3.19A-F), except for the correlation between BMI and E2, which showed a weak positive correlation in the MetSA group (Figure 3.19B).

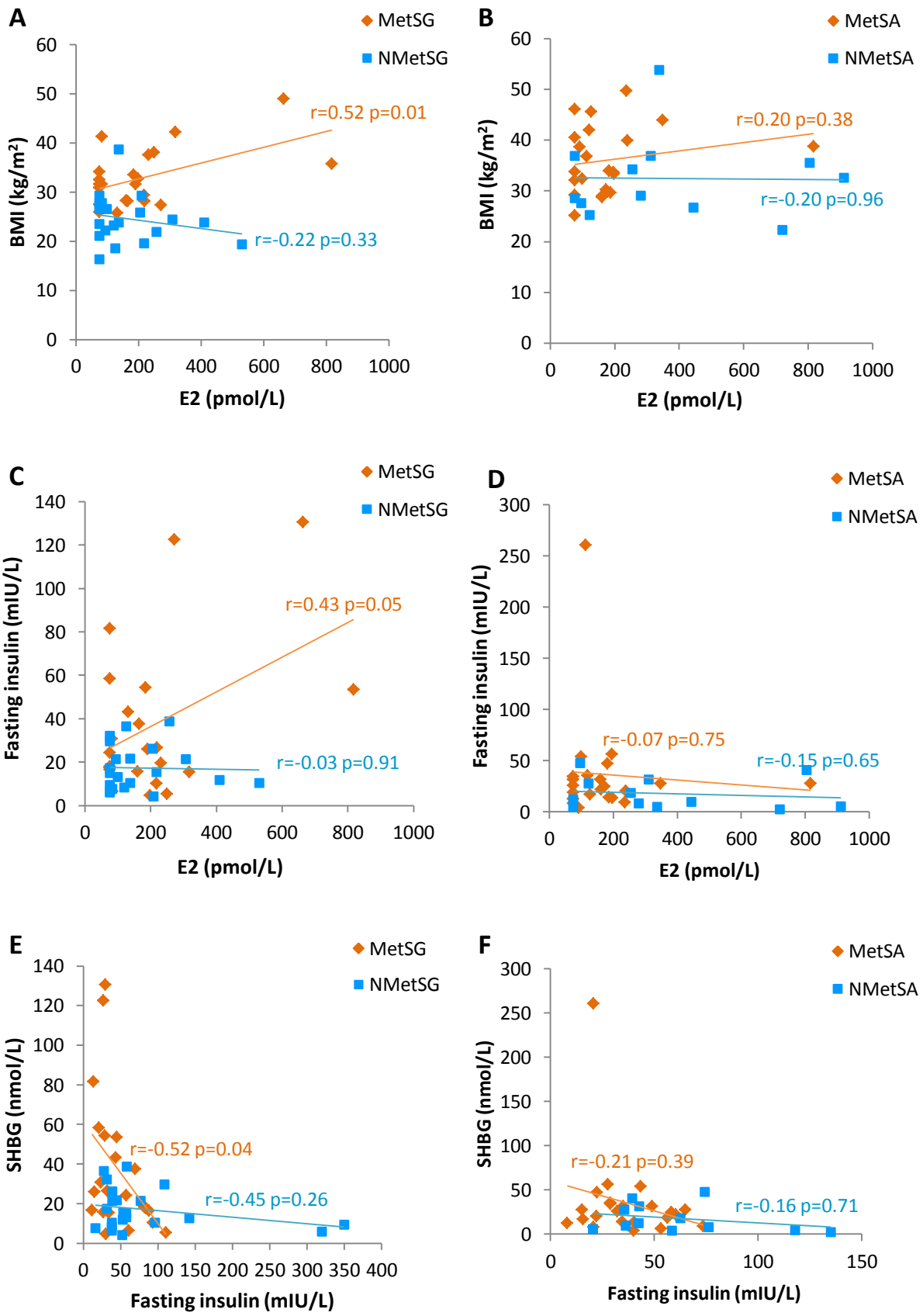


Figure 3.19: Correlations between the MetS and non-MetS groups for the gynoid and android body shape against E2 and BMI (A&B), E2 and fasting insulin (C&D), and SHBG and fasting insulin (E&F).

3.5.4 ABSI and selected parameters

Near similar results were obtained when correlations were drawn between ABSI and BMI for both the MetSG and MetSA, NMetSG and NMetSA groups respectively. Both the MetSG and MetSA groups displayed significant strong negative correlations (r=-0.48 for MetSG and r=-0.45 for MetSA, p=0.04 for both) (Figure 3.20A&B), whereas the non-MetS groups showed a near similar correlation coefficient, however, only the NMetSG group showing a statistical trend towards significance (r=-0.49, p=0.07). No significant correlations were evident for ABSI and IGF-1 in women of all the respective groups (Figure 34C&D).

Significant, strong positive correlations were also found between ABSI and TG in the women from the MetSA group (r=0.55, p=0.02), and between ABSI and LDL-c in women of the NMetSG group (r=0.78, p=0.02; very strong) (appendix IX).

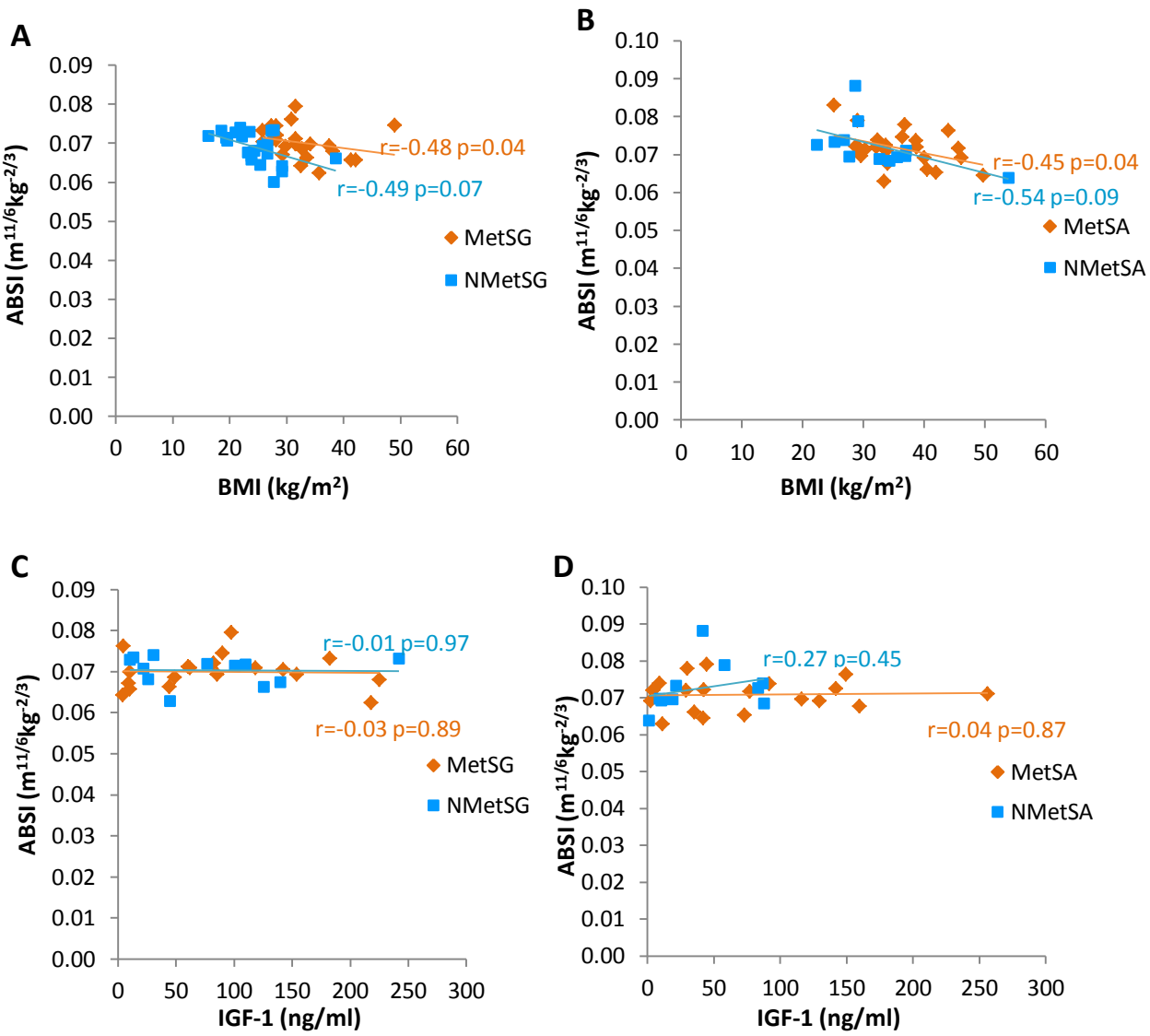


Figure 3.20: Correlations between ABSI: BMI, and ABSI: IGF-1 in the (A&C) gynoid and (B&D) android groups respectively.

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CHAPTER 4: DISCUSSION

4.1 Context of this chapter

This chapter argues the major findings of this current cross-sectional observational study. These findings include anthropometrical, BIA, physiological and biochemical blood parameters, and how it relates to the metabolic status, body shape and/or both, as well as possible cancer risk. Results within each sub-section will be discussed in a logical and integrated manner as described in Chapter 3.

4.2 Major descriptive findings of the study population

4.2.1 Overall high prevalence of obesity, the MetS and its individual components in women

The prevalence of the MetS is on the rise in most populations around the world (Tachang *et al*., 2012) and South Africa is no exception. Approximately 57.5 % of the women in the current study were characterized as having the MetS classified according to the IDF criteria (Figure 3.1), a prevalence which is considerably higher than what was previously reported in American (Bhanushali *et al*., 2013), Korean (Lim *et al*., 2011), European (Hu *et al*., 2004), Indian (Prasad *et al*., 2012), Australian (Cameron *et al*., 2007), and even in other South African (Motala *et al*., 2011; Peer *et al*., 2015[A]), as well as sub-Saharan African (Awosan *et al*., 2013; Tachang *et al*., 2012; Tran *et al*., 2011) populations. Although the prevalence of the MetS was significantly higher than that found in other parts of the world, our results confirmed the data obtained from another South African-based study which also made use of the IDF definition (Erasmus *et al*., 2012). Erasmus *et al*. (2012) found the prevalence of the MetS in Capetonian urban black women to be even higher (67.8 %) compared to our study. These discrepancies in the prevalence reported could be attributed to a number of factors. Firstly, the use of different definitions to characterize the MetS makes comparison between studies difficult. Our study population is somewhat similar to the population used by Erasmus *et al*. (2012), and the use of the IDF definition in our, as well as in Erasmus' study could have accounted for the high prevalence reported. This is in contrast to the relatively low prevalence (26.5 %) found in the study by Peer *et al*. (2015[A]) in which the JIS definition was used to categorize the sample.

Secondly, there are many confounding factors, including the region of interest, the population being studied (i.e. gender, age, ethnicity), the type of environment (i.e. urban or rural), as well as statistical adjustments (Prasad *et al*., 2012). Socio-economic status, sedentary lifestyles, level of physical activity, as well as genetic factors (Popkin *et al*., 2012) could also potentially have influenced the difference in prevalence. Although sample size might also impact the results (Prasad *et al*., 2012), this is not necessarily true for our population, since even with a small sample size (n=80), the prevalence

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of the MetS was significantly higher than that reported by other studies. For example, Peer *et al*. (2015[A]) had a relatively large sample size (n=1099 men and women), but still reported a relatively low prevalence of the MetS.

The clustering of none, one, two, three, four and five MetS risk factors were clearly dispersed between the MetS and non-MetS groups, with the majority of the MetS participants displaying three risk factors (57 %) compared to the three percent reported in the non-MetS counterparts (Figure 3.3). What is somewhat worrisome is that 15 % of the MetS group presented with all five risk factors. Another concern was that only 18 % of participants without the MetS presented with no risk factors. This number was alarming low, since studies have shown that the MetS and its risk factors have all been independently associated with CVD and T2DM (Alberti *et al*., 2009; Beltrań-Sánchez *et al*., 2013; Kaur, 2014). This therefore suggests that even the women in our group with one risk factor could be at risk for CVD, however, it is still not entirely clear whether the MetS, or the sum of its individual risk factors are necessarily superior in determining cardiovascular risk.

The current study found that abdominal obesity (measured by WC) (73.8 %), elevated blood pressure and decreased HDL-c (both 68.8 %) were the more prevalent MetS risk factors (Figure 3.4 & 3.5), which are in agreement with other South African studies. Peer *et al*. (2015[A]) showed that abdominal obesity (86 %) and decreased HDL-c levels were the most prevalent MetS risk factors in a black female population from Cape Town, while Motala *et al*. (2011) confirmed a high prevalence of decreased HDL-c levels in women. The high prevalence of elevated blood pressure in the current study contradicts previous studies that have found the prevalence of elevated blood pressure to be significantly higher in men (Erasmus *et al*., 2012; Motala *et al*., 2011; Peer *et al*., 2015[A]). Although this might be true, various studies have proposed that central obesity might be responsible; either independently, or through the induction of insulin resistance (*via* inflammatory mediators released from adipose tissue); for the development of systemic hypertension and dyslipidaemia (Alberti *et al*., 2009; Fuentes *et al*., 2013; Zhao & Liu, 2013; Mendonça *et al*., 2015). Since the women in our study had an increased WC, this explanation might also hold true for our population.

Other possible risk factors that might explain elevated blood pressure includes an unhealthy diet (calorie dense food, high salt intake, and low fruit and vegetable intake), lack of physical activity, as well as excessive alcohol consumption, and tobacco use (Peltzer & Phaswana-Mafuya, 2013; Ulasi *et al*., 2011). Smoking, alcohol consumption and physical activity levels of women in this current study could possibly explain the high prevalence of high blood pressure (Appendix X). Although we did not classify participants as heavy smokers or alcohol drinkers, approximately 56 % of our total study population were smokers, either previous smokers (20 %) or current smokers (36 %). In addition, 80 % of the population were also classified as alcohol drinkers, either previously (25 %), or currently (55 %) (Appendix X). Lastly, only a small proportion of the participants performed vigorous intensity

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work (17.5 %) and recreational activity (7.5 %), whereas more participants partook in moderate intensity work (51.25 %), and recreational activity (71.25 %) (Appendix X). This therefor showed that more participants participated in a moderate level of physical activity.

Although BMI is not normally used to indicate abdominal obesity (according to the IDF definition), various studies have linked the high prevalence of the MetS with a higher prevalence of overweight/obesity in South African women (Erasmus *et al.*, 2012; Motala *et al.*, 2011; Peer *et al.*, 2015[B]). The current study found an overall higher prevalence of overweight (31 %, n=14 *vs* 47 %, n=16), and obesity (67 %, n=31 *vs* 21 %, n=7) in both the MetS and non-MetS groups (Figure 3.2), which collectively accounts for a prevalence of 85 % (n=68). These results corroborated the findings by Peer *et al.* (2015[B]), in which the prevalence of overweight/obesity (BMI $\geq$ 25 kg/m^2) was shown to be 82.8 %, the highest reported prevalence of overweight/obesity in sub-Saharan Africa at the time. van Zyl *et al.* (2012) also showed that the prevalence of overweight/obesity in females is high (43.1 %), although not as high as in our population. Taken together, obesity is a major problem in South African females and may predispose individuals to develop the MetS if it coincides with other MetS risk factors.

In order to determine the effect of adipose tissue distribution on the metabolic status, females were grouped according to body shape. Males normally store adipose tissue in the abdominal region of their body, displaying an android body shape, while females store adipose tissue in the gluteo-femoral region, displaying a gynoid body shape (Patidar, 2013; Stewart & Sutton, 2012). Women only store a small percentage of their TBF in their abdominal area; however this percentage is significantly increased when a moderate level of obesity is reached (Peppa *et al.*, 2012). This finding of an increased prevalence of android body shape in overweight and obese women, as well as women with the MetS, was also supported by Sardinha *et al.* (2012). Our data is in accordance with previous studies of an increased prevalence of an android body shape in women with the MetS compares to those without the MetS (MetSA, 50 % *vs* NMetSA, 35 %) (Figure 3.1B&C). Several studies relate these findings to changes in urbanization, nutrition transition, economy and lifestyle, i.e. unhealthy diets, sedentary behaviour and lack of physical activity (Bourne *et al.*, 2002; Dias *et al.*, 2014; Erasmus *et al.*, 2012; Steyn *et al.*, 2012; Vorster *et al.*, 2005; Vorster *et al.*, 2011), all of which have been shown to significantly impact lifestyle diseases, such as obesity and hypertension, ultimately resulting in a high prevalence of the MetS. We speculate that our findings may also in part be due to cultural beliefs, since unhealthy eating habits in some African cultures, and therefore a large BM is accepted and associated with a higher social status (Mciza *et al.*, 2005).

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4.3 Descriptive findings according to metabolic status

4.3.1 Anthropometric and BIA findings: women with the MetS displayed an exacerbated body composition profile

Anthropometric and BIA results revealed significantly higher BM, BMI, WC, HC, WHR and FM (%) in the MetS groups compared to their non-MetS counterparts, while FFM (%) was significantly lower (Table 3.1). Sigdel *et al*. (2014) was also able to show that BMI and WHR was significantly higher, whereas, Liu *et al*. (2013) found not only BMI, but also WC, WHR and FM (%) to be significantly elevated. Our findings of an increase in FM (%) and decrease in FFM (%) have also been supported by Park & Yoon (2013). Namwongprong and colleagues (2014) also concurred, but additionally also reported a higher BM.

Peer *et al.* (2015[B]) showed that BMI, WC and WHR can predict the presence of the MetS, as well as its individual components in a South African population. Our findings adds value since the women in our study were predominantly obese (increased BM, FM and BMI), and displayed both android (WC and WHR), and gynoid body shapes (HC), which may play a role in the pathophysiology of the MetS, and may therefore be predictors of the MetS. On the other hand, Wang *et al*. (2009) showed that an increase in FM and decrease in FFM was also associated with a significantly higher risk for the MetS.

During a positive energy state for example, adipose tissue stores excess energy in the form of TG in adipocytes, which in turn leads to hyperplasia, as well as hypertrophy, and ultimately to an increase in fat mass (Bjørndal *et al*., 2011; Jung & Choi, 2014). Alternatively, a change in FFM may lead to a change in insulin sensitivity and glucose disposal, and in that way contribute towards the development of the MetS and its components (Peppa *et al*., 2012; Zhang *et al*., 2015). Since high insulin exert catabolic effects on muscle mass (Peppa *et al*., 2012; Zhang *et al*., 2015), a decrease in muscle mass may also be considered a risk factor for the development of metabolic diseases and cardio-metabolic risk (Park & Yoon, 2013; Tanaka *et al*., 2012). However, muscle mass did not differ between the MetS and non-MetS groups in our study, which suggests that other factors might have accounted for these discrepancies. The main criticism of the study by Park & Yoon (2013) was that the unequal distribution of participants within the MetS and non-MetS groups– the non-MetS group had approximately ten times the number of participants compared to the MetS group. They also included male participants and participants were from a different ethnicity, and a different definition of the MetS.

The body shape index, ABSI, did not show any differences, a finding that is supported by Haghighatdoost and colleagues (2014). They showed that ABSI was a weak predictor of both CVD risk, as well as the MetS itself (Haghighatdoost *et al*., 2014). Although ABSI was proposed to be a more reliable measure of body fat distribution *vs* traditional anthropometric indices, such as BMI and

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WC (Krakauer & Krakauer, 2012), this is not the case for the current population. To our knowledge this was also the first South African study to report findings on ABSI in women with or without the MetS.

4.3.2 Physiological and blood parameters: women with the MetS displayed a deregulated metabolic and sex hormone profile

The MetS consists of, and is characterized by, several risk factors including obesity (abdominal obesity), dyslipidaemia, elevated arterial blood pressure, IR/hyperinsulinaemia and impaired glucose homeostasis (Alberti *et al*., 2009; Deprès *et al*., 2008; Kassi *et al*., 2011; Kaur, 2014; Okafor, 2012). Although the exact pathophysiology behind the MetS is still unclear, and several mechanisms have been proposed (Kassi *et al*., 2011), all studies are in agreement of the core factors involved in the development of the MetS (Kassi *et al*., 2011).

In this current study, the women in the MetS group showed significantly higher SBP, DBP, FBG, fasting insulin, LDL-c and TG levels, whereas HDL-c levels was significantly lower, as well as TC was shown not to be significantly different compared to the non-MetS group (Table 3.2). These findings were corroborated by Liu *et al.* (2013) for all the mentioned parameters with the exception of insulin and LDL-c. Various other studies showed different combinations of the physiological and blood parameters found in the current study. Momesso *et al*. (2011) showed a significantly higher blood pressure, TG and decreased HDL-c in the MetS *vs* non-MetS groups. Total cholesterol was also unchanged in the study by Motala *et al.* (2011), which supports our findings, and it was suggested that this might be so due to it being a weak predictor of CVD and the MetS in both men and women (Karalis, 2014). Although TC was shown to be unchanged in all the studies mentioned thus far, Sigdel *et al*. (2014) showed TC, TG, LDL-c and insulin levels to be significantly higher in the MetS group.

The MetS is also associated with a deregulated sex hormone profile (Sinicrope & Dannenberg, 2011). The women in the MetS group showed significantly higher FAI while, SHBG levels were significantly lower (Table 3.2). These results are supported by a relatively old study from Weinberg *et al*. (2006), in which increased T and FAI and decreased SHBG levels were observed. However, a more recent study by Ziaei & Mohseni (2013) reported similar results. Both these studies suggested that the altered sex hormone profile may be ascribed to obesity and IR/hyperinsulinaemia, which in turn can decrease E2 production (by inhibiting aromatase enzyme gene expression) and increase FAI by directly decreasing SHBG levels (Suba, 2012). This is a quite likely mechanism, since the fasting insulin levels were also significantly higher in the MetS group in the current study.

Although no significant differences were observed for E2 and female testosterone, it might not necessarily indicate that the above mentioned mechanism may not be partly involved in the differences observed in the sex hormone profile. No statistically significant differences were observed

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for CRP and IGF-1 in our study. Reasons for this finding might be explained by the specificity of the IGF-1 and CRP kits that were used, as well as the large variation in IGF-1 and CRP levels found in these women. Other reasons could include the small sample size which would have affected the statistical power of this study.

4.4 Descriptive findings according to body shape

4.4.1 Anthropometric and BIA findings: women with an android body shape displayed an exacerbated body composition profile

Evidence shows that body shape classification, also known as fat pattering, rather than TBF % has a stronger clinical significance on the development of CVD; T2DM, MetS as well as the risk of cancer development (Eston & Reilly, 2009; Momesso *et al*., 2011; Kabat *et al*., 2015; Kim *et al*., 2007). The women with an android body shape in the current study were significantly older (Table 3.3), which is supported by a higher prevalence of android body shape found in post-menopausal *vs* pre-menopausal women, since post-menopausal women are in general older (Mastaglia *et al*., 2012; Noroozi *et* al., 2010). Also, women with android body shape displayed a significantly exacerbated body composition profile (increased BM, BMI, WC, HC, WHR and ABSI) compared to the women with a gynoid body shape. Muscle mass was also found to be significantly higher in the android body-shaped women. In contrast, no difference was observed for FM (%) and FFM (%). These results confirmed the findings by Alwachi *et al*. (2013) who showed that android body shape is associated with an increase in age, BM, BMI, WC, HC and WHR. Orbetzova *et al*. (2012) showed that body shape did not alter BM, BMI, WHR, FM (%) and FFM (%); however, compared to an age-matched control group, significant differences in FM (%) and FFM (%) were evident. These observed differences could be due to the fact that Orbetzova *et al.* (2012) included an age-matched control group, and only included obese women in their study.

Because the android body shape is mainly characterized by the presence of a large amount of storage fat in the abdominal area (Patidar, 2013), it is not uncommon to find an increase in BM, BMI, and WC. These anthropometric indices increase as a result of the increase in VAT (WC and WHR), as well as subcutaneous adipose tissue (SAT) (HC) (Peppa *et al*., 2012). These body composition changes, especially those associated with VAT can therefore also increase the risk for CVD, T2DM, the MetS and the risk of cancer development (Cornier *et al*., 2011; Namwongprom *et al*., 2014).

A surprising finding from the current study was the higher muscle mass, evident in the android body shape group. This higher muscle mass in women with android body shape may be a compensatory mechanism in order to sustain a greater body weight. However, obesity, and fat deposition has been suggested to negatively impact muscle tissue (Koo *et al*., 2015), and was also negatively associated with BMI, WC, SAT and FM after adjustment for age (Hamasaki *et al.*, 2015; Koo *et al.*, 2015; Zhang

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et al., 2015), possibly through the catabolic effects of insulin on muscle mass. It is also still unknown whether general adiposity or regional adiposity has an effect on muscle health, and this may be in part due to the fact that research in this field has extensively focused on the elderly population (Koo *et al.*, 2015).

We observed that body shape might have the ability to predict ABSI, i.e. women in the android body shape group showed significantly higher ABSI compared to their gynoid counterparts. This observation is supported by the significant positive association between ABSI and abdominal obesity found by Krakauer & Krakauer. (2012). High ABSI values therefore correlated with high WC, and this in turn, corresponds to a larger abdominal area and increased VAT (Krakauer & Krakauer, 2012).

4.4.2 Body shape alone does not predict changes in physiological or biochemical blood parameters in women

Android obesity, characterized by an increase in VAT, is normally associated with a worsened metabolic risk factor profile (IR, hypertension, dyslipidaemia and inflammation) and seemed to be an underlying factor in the pathophysiology of various diseases including the MetS and cancer, while gynoid obesity, characterized by an increase in SAT, is thought to be cardio-protective, however its relationship with disease risk is still controversial (Orbetzova *et al*., 2012; Suba, 2012; Westley & May, 2013).

No significant differences were found for any physiological or biochemical blood parameters between android and gynoid groups (Table 3.4). These findings contradict the results from previous studies investigating the effect of android and gynoid body shape on various metabolic-associated and other biochemical blood parameters. These studies showed that an android body shape is associated with a worsened physiological and biochemical profile (Momesso *et al*., 2011; Namwongprom *et al*., 2014; Simó *et al*., 2015; Wiklund *et al*., 2008). We speculate that these discrepancies may be due to the following: Firstly, our study had a relatively small sample size, which could have affected the statistical power, and therefore did not reveal differences. Secondly, somatotyping was used as classification method in contrast to the use of gold standard methods, i.e. DEXA, CT, MRI and other anthropometric indices (WC and WHR) (Eston & Reilly, 2009; Norton & Olds, 1996; Rothney *et al*., 2009; Stewart & Sutton, 2012). Thirdly, since the women in both the android and gynoid groups had relatively high BMI, this could have accounted for the lack of differences in blood parameters as seen in this study. Lastly, body shape in relation to body composition and metabolic health has mostly been studied in menopausal women (Mastaglia *et al*., 2012; Noroozi *et* al., 2010), and women with hyperandrogenism (Simó *et al*., 2015), and not in apparently healthy female farm workers, such as this population.

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4.5 Major findings of study population according to both metabolic status and body shape

Various significant and interesting findings were observed when both body shape and metabolic status of the study population were taken into consideration.

4.5.1 Metabolic status showed effects on certain anthropometric and BIA measurements in women with either gynoid or android body shape's

Irrespective of body shape, BM and BMI were significantly higher in both the MetS groups' *vs* the non-MetS groups (Figure 3.6A & 3.8A). This finding is well supported by previous studies, and is considered as a causal factor in the pathophysiology of the MetS (Liu *et al*., 2013; Namwongprong *et al*., 2014; Peer *et al*., 2015[A]; Sigdel *et al*., 2014). Hip circumference was also shown to be significantly higher in the MetSG group *vs* the NMetSG group (Figure 3.7A). Although no differences were observed between the MetS and non-MetS groups in relation to body shape, a significant difference was evident in the NMetSG *vs* NMetSA groups for WHR (Figure 3.7B). This finding is well supported by studies showing a higher VAT content in the android body's shape *vs* the gynoid body shape group (Bjørndal *et al*., 2011). Females normally store adipose tissue in the gynoid region and only store a small percentage of their TBF in their abdominal area; however this percentage is significantly increased when a moderate level of obesity is reached (Patidar, 2013; Peppa *et al*., 2012; Stewart & Sutton, 2012).

No significant differences were observed for ABSI between women of all respective groups, similar to that observed when only metabolic status was taken into consideration (Figure 3.8B). Similar reasons could therefore account for the observation here as seen in section 4.3.1. Since ABSI has been proposed to be a more reliable measure of body fat distribution (Krakauer & Krakauer, 2012), and we observed equal participant distribution within the MetS and non-MetS groups with regards to body shape, it could explain the "no difference" observed. The participants in the MetSG group had a significantly higher FM (%) and significantly lower FFM (%) compared to the NMetSG group, thus indicating an effect of metabolic status (Figure 3.9A&B). Our findings are supported by evidence showing an increase in FM (%) in women with gynoid obesity *vs* age-matched controls (Orbetzova *et al*., 2012), and increase in FM (%) and decrease FFM (%) in the MetS (Park & Yoon, 2013). No significant differences were evident for muscle mass (Figure 3.9C), but this is not surprising since an increase in FM does not necessarily correlate with a difference in muscle mass.

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4.5.2 Metabolic status showed effects on MetS risk factor measurements in women with either gynoid or android body shapes

The literature indicates strong relationships between all the components of the MetS and fat storage distribution. This association is proposed to be a result of an increased VAT, and its associated dysfunction in the abdominal area (Bjørndal *et al*., 2011). However, the role of gynoid obesity (increased SAT) has also been implicated in the development of individual MetS risk factors (Patel & Abate, 2013).

Waist circumference (reflective of an increased VAT) was significantly higher in the NMetSA group *vs* the NMetSG group (Figure 3.10A), which is supported by evidence from Bjørndal *et al*. (2011). A higher WC was also evident in the MetSG *vs* NMetSG women, which can be explained by an increase in SAT in the abdominal area in the MetSG group (Patel & Abate, 2013).

No significant differences were observed for FBG between women of all respective groups (Figure 3.10B). A possible explanation for this might be related to general obesity, irrespective of its anatomical distribution and metabolic status. However, BM and BMI were significantly different between the respective MetS groups and their non-MetS counterparts (Figure 3.6A & 3.8A). Waist circumference was also shown to be significantly different, not only between the MetSG and NMetSG group, but also between the gynoid and android groups of the non-MetS group. Thus, although obesity cannot elucidate this occurrence, adipokine deregulation (i.e. CRP), could possibly explain this occurrence, since a dysregulation in the synthesis of these adipokines and their actions in relation to obesity may lead to hyperglycaemia (Lasselin *et al*., 2014). No significant difference was evident for CRP therefor, no definitive conclusion could be drawn and the relationship between the MetS, body shape and FBG remains elusive.

We found a significant difference of metabolic status in both the gynoid and android body shape groups for blood pressure (Figure 3.10E&F). Significantly higher results were obtained for both SBP and DBP in the MetS group's *vs* the non-MetS groups. This is supported by evidence showing that the MetS is associated with an increased arterial blood pressure (Okosun *et al*., 2015; Yanai *et al*., 2008), which is also proposed to be directly due to IR and abdominal obesity (increased VAT) (Yanai *et al*., 2008). This is likely since BM and BMI (Figure 3.6A & 3.8A) were significantly different between the MetS and its respective non-MetS groups.

Obesity has been shown to have an effect on several factors that influence blood pressure, i.e. volume expansion and peripheral resistance (Daniels, 2012). Obesity-related insulin resistance/hyperinsulinaemia increases blood pressure by activating the sympathetic nervous and the renin angiotensin aldosterone system (RAAS), leading to an increase in sodium reabsorption by the kidneys, resulting in renal vasodilation and volume expansion, and ultimately leads to the

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development of hypertension (Yanai *et al*., 2008). In addition, it has been shown that obesity-associated hypertension, may also be due to increased peripheral resistance which ultimately leads to elevated blood pressure (Daniels, 2012). Similar reasons could also account for the observation here as seen in section 4.2.1.

Dyslipidaemia is a characteristic of the MetS and also a risk factor for CVD and atherosclerosis (Okafor, 2012). The pathophysiology of the MetS directs to obesity (abdominal obesity (adipose tissue dysfunction)) and IR as the underlying factors in its pathophysiology (Kassi *et al*., 2011; Kaur, 2014). Women with the MetS, especially those with abdominal obesity, show a more anthrogenic lipid profile and this may explain their greater CVD risk (Liu *et al*., 2013; Momesso *et al*., 2011; Sigdel *et al*., 2014). Android obesity and IR have been shown to be associated with a combination of dyslipidaemia characteristics (TG, HDL-c and LDL), and it is more likely to occur in this combination (Karalis, 2014). No differences were observed for HDL-c in the android groups, but a significant difference was observed for TG (Figure 3.10C&D). Although our results contradicts what has previously been reported for android obesity and its association with dyslipidaemia, the small sample size in the NMetSA group (n=12) could possibly explain these findings. Lower HDL-c and elevated TG levels were also evident in the MetSG *vs* NMetSG group. This is supported by evidence showing that the MetS is characterized by dyslipidaemia (Liu *et al*., 2013) and that GFM shows a positive association with TG and negative with HDL-c (Okosun *et al*., 2015).

In summary, most previous studies investigating metabolic disease risk, android and gynoid body shapes, considered a normal BMI or either adjusted for BMI. In addition, the previous studies also only compared android to gynoid body shapes, and in most cases did not include a control group. In our study the metabolic status and body shape were both shown to have a high prevalence of overweight and obesity. We thus speculate that when a certain BMI is reached, body shapes might not necessarily have an effect on certain anthropometric, blood and BIA parameters. Recent evidence shows that both android and gynoid fat mass increases with the prevalence of cardio-metabolic risk factors and that the combination of increased android and gynoid fat mass is associated with increased odds of developing MetS risk factors compared to AFM and GFM alone (Okosun *et al*., 2015). Thus, the A/G ratio has been suggested to be a better predictor of the MetS.

Although TC and LDL-c are not considered as diagnostic criteria for the MetS, participants with the MetS have been found to display increased TC and LDL-c levels (Sigdel *et al*., 2014). Low density lipoprotein-cholesterol and TC did not differ in our study (Figure 3.11A&B). One specific study also showed that even though the other lipid levels may have been altered as a result of the MetS and body shape, TC can stay within its normal range (Karalis, 2014), which has also been shown in our study.

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4.5.3 Insulin-IGF-1 axis: metabolic status and body shape combined does not predict changes of fasting insulin and IGF-1 in women

High IGF-1 levels have been associated with an increased risk for cancer development (Arcidiacono *et al*., 2012); however, current evidence on IGF-1 in relation to metabolic disease states is still very controversial. For example, low levels of IGF-1 have been suggested to have beneficial effects on glucose homeostasis and may also sensitise insulin actions, thereby decreasing metabolic disease risk, while high levels have been associated with an increased risk for cancer development (Kabir *et al*., 2010).

Low levels of IGF-1 have been suggested to be associated with an increased risk for the MetS. In a study by Oh *et al*. (2012), IGF-1 levels were significantly lower in the MetS *vs* non-MetS participants, with IGF-1 being lowest in participants with the most MetS risk factors. The authors proposed that IR and hyperinsulinaemia may induce GH receptor resistance and thus decrease IGF-1 levels (Oh *et al*., 2012). On the other hand, Friedrich *et al*. (2013) showed that participants with the MetS had significantly higher IGF-1 levels *vs* healthy age-matched controls.

Two mechanisms are proposed to help explain the elevation in IGF-1 as a result of hyperinsulinaemia/IR. Directly, IGF-1 can increase by increasing hepatic GH receptor expression and thus the production of IGF-1 in the liver (Hursting *et al*., 2012). Indirectly, through the down regulation of IGF-BP production by reducing hepatic gene expression and protein synthesis of IGF-BP, this in turn can increase the bioavailability of IGF-1 (Arcidiacono et al., 2012). Additionally, adipocytes itself can secrete IGF-1, which may explain the increase in IGF-1 (Hursting *et al*., 2012). Studies on IGF-1 and the MetS are however limited and various reasons could explain these discrepancies. The sample sizes, ethnic differences, selection bias, the study design and studies measuring either free IGF-1 or total IGF-1 can all be confounding factors. Studies on body composition, and body shape in relation to IGF-1 levels are however also limited.

In the current study, no significant differences were observed for fasting insulin or IGF-1 between any of the women in their respective groups (Figure 3.12A&B). Our findings are in agreement with findings of Kabir *et al*. (2010), who found no difference in IGF-1 levels in participants with metabolic disturbances *vs* matched controls. This suggests that although free IGF-1 may change due to adiposity, total IGF-1 remains within its reference range (Renehan *et al*., 2006), and therefore remain unchanged. Another reason for the observation of "no difference" could include the specificity of the human IGF-1 ELISA kit. Seventeen participants were excluded because their IGF-1 levels were below detection limit (< 0.1 ng/ml), whereas two participants were excluded because their IGF-1 was extremely high. Age, gender, ethnicity and obesity have also been shown to influence IGF-1 levels (Gram *et al*., 2006). It should however be stressed that no accurate conclusion could be drawn linking the MetS, body shape and cancer risk with the data obtained from this study.

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4.5.4 Inflammation: both metabolic status and body shape does not predict changes in CRP

The role of inflammation as an underlying factor in the pathophysiology of metabolic conditions (obesity and the MetS) has been extensively studied, but has recently also been implicated in risk of cancer development (Cefalu, 2009; Shrivastava *et al*., 2015). An increase in inflammatory biomarkers in obesity (Kao *et al*., 2009), android body shape (Alwachi *et al*., 2013) and the MetS (den Engelsen *et al*., 2012) have been well documented. Evidence for an increased BMI and WC in relation to increased CRP levels has also been shown (Aronson *et al*., 2004; Kao *et al*., 2009). However, the specific mechanism responsible for the pathophysiology linking the MetS and inflammation still needs to be elucidated. One proposed mechanism states that an increase in adipose tissue (in obesity and the MetS) as a result of adipose tissue dysfunction (hypertrophy, hyperplasia and adipokine deregulation) can lead to an increase in IL-6 synthesis. This in turn regulates hepatic CRP-synthesis and thereby increases CRP levels (Braun *et al*, 2011; Choi *et al*., 2013). Also, adipose tissue itself may be a source of CRP (Braun *et al*, 2011).

The current study contradicts the above evidence as we found neither metabolic status, nor body shape to have an effect on CRP levels (Figure 3.13), even though clear differences were observed for almost all anthropometric and BIA measures of obesity. Reasons for this finding may include the relatively small sample size, which combined with the large variation in CRP levels could have accounted for no difference found. Literature has highlighted differences in CRP levels in relation to the MetS and body shape. For example, mean CRP levels in participants with the MetS with android body shape were reported to be 1.4 mg/L (Vidyasagar *et al*., 2013), mean CRP levels of 5.39 mg/L in post-menopausal females with android body shape (Alwachi *et al*., 2013), and mean CRP levels of 1.96 mg/L in healthy participants with android body shape (Lapice *et al*., 2009). These studies therefore highlight the differences in CRP levels even when measured in women with the same body shape. In addition, the human CRP ELISA kit that was used could not detect any CRP levels below 15 pg/ml, therefore excluding n=14 individuals. Two women were also excluded as their levels were extremely high and deemed as outliers and were subsequently removed prior to analysis.

Although no significant differences were evident between any of the groups, we did however observe the mean CRP levels in the MetS groups to be greater than 10 mg/L, similar to what has already been reported in the literature (Aronson *et al*., 2004; Ishii *et al*., 2012). This level is suggestive of an underlying infection or low-grade inflammation. Evidence shows that CRP levels greater than 10 mg/L in obesity are not uncommon and have been documented (Aronson *et al*., 2004; Ishii *et al*., 2012). Furthermore, both studies have also shown that people with a more severe class of obesity, have higher CRP levels beyond this reference range, which suggests that CRP levels greater than 10

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mg/L is associated with overweight and obesity. It should however be noted that inflammation could also have contributed considerably.

Since the metabolic status and body shape did not alter CRP levels, its effect still warrants further investigation, especially with regards to the link between the MetS, inflammation and risk of cancer development.

4.5.5 Sex hormone profile: metabolic status and body shape in combination does not predict changes in E2, female T, SHBG and FAI

Evidence on the role of sex hormones in the pathophysiology of certain disease states (obesity, IR, CVD, the MetS and the risk of cancer development), and its associated risks are still lacking, despite the fact that there is convincing evidence of a casual role of sex hormones in the development of these diseases of lifestyle (Torréns *et al*., 2009). Furthermore, it seems that oestrogen might also play a pivotal role in these disease states (Suba, 2012).

The MetS is characterized by an altered sex hormone profile, i.e. increased oestrogen and an increase in androgen excess (increased female T, FAI and decrease in SHBG) (Calle & Kaaks, 2004; Korhonen *et al*., 2003; Weinberg *et al*., 2006; Ziaei & Mohseni, 2013). An increase in E2 levels and androgen excess is also associated with an increased cancer risk in women (Westley & May, 2013). In contrast, androgen excess (female T, FAI and decrease in SHBG) and E2 deficiency have been associated with an increase in VAT and an android body shape, both in pre-and post-menopausal women, which increases the risk to develop CVD and the MetS (Gravena *et al*., 2013; Kim & Halter, 2014; Momsesso *et al*., 2011; Weinberg *et al*., 2006). These findings highlight the controversies around the role of sex hormones in the development of certain diseases such as lifestyle associated cancer and other diseases of lifestyle.

Changes in E2 levels in the body also influence the risk and development of the MetS by influencing its individual components. For example, E2 has regulatory effects on lipid and glucose metabolism, it also has been associated with hypertension, as well as IR (Brand *et al*., 2011; Suba, 2012), and have been shown to increase during obesity (Kalyani *et al*., 2009). In addition, both E2 and female T levels can be increased due to a decrease in SHBG levels due to obesity-associated hyperinsulinaemia (Folkerd & Dowset, 2010; Mendonça *et al*., 2015; Westley & May, 2013).

We found no significant differences for E2 in all the women of the respective groups (Figure 3.14), and therefore the effect of metabolic status and body shape on E2 levels remains inconclusive. We conclude that the variation in E2 levels reported between the different groups might be related to the changes in E2 concentrations during the different phases of the menstrual cycle. We could not establish in what menstrual phase each of the women were, and thus could not establish if E2 levels were lower, within range, or higher than the normal physiological levels for each individual.

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Approximately 18.75 % (n=15) of the total population of women were found to have primary or secondary hypogonadism, which might suggest menopause, ovarian failure, birth control usage, pituitary or hypothalamic dysfunction (Fraietta *et al*., 2013). However, further investigation with appropriate study design is needed to warrant this finding. Although E2 is known to increase during obesity, and clear differences were observed in anthropometric and BIA parameters in our study; glucose, as well as insulin levels were not different, which could have resulted in "no difference" observed in E2 levels.

As a result of obesity, and its association with hyperinsulinaemia, both E2 and female T can increase with a concomitant decrease in SHBG. However, no differences were observed for T, SHBG, as well as FAI (Figure 3.15 & 3.16). Furthermore, women in all four groups displayed mean female T levels within normal physiological range (Figure 3.15A). This could be explained by the fact that female T is produced in very small quantities in women, by the ovaries and adrenal glands (Al Kindi *et al*., 2010). In addition, the deregulated adipokine profile, as a result of obesity, may also be responsible for the low female T levels. For example, obesity is associated with an increase in leptin levels, and this in turn can interfere with luteal hormone stimulation of androgen synthesis, resulting in a decrease in female T production, which may explain why female T levels were below normal physiological cut-off value (Salam *et al*., 2012). Previous studies have however shown that female T is increased in the MetS, but didn't show that it was above the normal physiological levels (Momsesso *et al*., 2011; Weinberg *et al*., 2006). Since no differences were observed for insulin and glucose, and both are known to affect the sex hormone profile, it might help explain that "no differences" were observed in any of the sex hormones measured. The results for FAI are conflicting and limited since FAI has not been studied in apparently healthy women in this specific setting. Other confounding factors could include age, menopausal status, obesity, thyroid dysfunction, ovarian failure, polycystic ovarian syndrome, as well as sample size (Wallance *et al*., 2013).

The effect of both metabolic status and body shape on sex hormone levels remains inconclusive since no differences were observed. Furthermore, no immediate conclusions could be drawn to describe the link between the MetS, sex hormones and cancer risk.

4.6 Correlation analysis findings

4.6.1 Obesity, body shape and adipose tissue's relationship with growth factor (IGF-1), metabolic status and cancer risk

Adipose tissue distribution seems to play a role in the pathophysiology of the MetS and cancer risk by having an effect on growth factors (Arcidiacono *et al*., 2012). Literature suggests that

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hyperinsulinaemia/IR and increased IGF-1 levels increases cancer risk by affecting downstream signalling pathways regulating mitogenesis, apoptosis and angiogenesis (Hurstings *et al.*, 2012; Ramos-Nino, 2013). Correlation analysis revealed significant associations between IGF-1 and selected anthropometric and BIA parameters (Figure 3.17).

Correlation analysis for IGF-1 and WHR revealed a positive relationship in all four study groups (Figure 3.17A&B), however, only the NMetSG group' correlation coefficient showed significance. This finding was somewhat unexpected, since theoretically, android body shape, characterized by a higher WHR, should result in an increase in IGF-1 levels. These discrepant findings may be explained by the controversial role of IGF-1 in disease states. Alderete *et al.* (2010) reported a negative association between IGF-1 and VAT (android body shape), while Schmitz *et al.* (20110) showed IGF-1 to be negatively correlated with BMI and WHR. These differing outcomes suggest that in a normal overweight person with android body shape (NMetSA in our scenario), as WHR increases, IGF-1 should decrease. However, this was not evident in our study population which revealed positive relationships.

Literature also states that increased insulin levels, which cause a decrease in IGF-BP, can thereby indirectly increase IGF-1 levels (Arcidiaconco *et al.*, 2012). Insulin-like growth factor-1 levels will increase in relation to a certain level of obesity, but with greater adiposity, this increase will induce a negative feedback mechanism on GH secretion, and this will result in a decrease in IGF-1 (Gram *et al.*, 2006). This could therefore suggest that the level of adiposity necessary to decrease IGF-1 has not been reached in our different study groups. Body mass index, could explain these findings, since a higher BMI was observed in both the MetSA and MetSG groups (Figure 3.8A), which relates to the almost negligible correlations found between IGF-1 and WHR. The BMI for both NMetSG and NMetSA were significantly lower than that of their respective MetS groups, with the gynoid group here displaying a higher BMI and therefore a stronger positive correlation.

However, BMI and WHR only confirms body shape and obesity, but it does not inform about the adipose tissue *per se*. Correlations between FM (%), FFM (%) and IGF-1 or insulin might be more informative. Correlation analysis between IGF-1 and FM (%), and, IGF-1, and FFM (%) revealed opposite findings (Figure 3.17C-F), with only the android body shape groups indicating significant correlations. For IGF-1 and FM (%), all group correlations were positive, except for the NMetSA group. The opposite was true for the correlation between IGF-1 and FFM (%). This is not unexpected since a person with a high FM (%), normally has a lower FFM (%), possibly explaining these contrasting associations (Park & Yoon, 2013). Interestingly, even though insulin or IGF-1 did not differ between the different study groups, both the MetSA and NMetSA groups' correlations were significant.

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The positive associations between IGF-1 and FM (%), and the negative associations between IGF-1 and FFM (%) may be explained by obesity since adipocytes secrete IGF-1 (Hursting *et al*., 2012. Furthermore, since more storage fat is normally disturbed around the abdomen (Patidar, 2013), it could account for the significant positive correlation observed in the MetSA group. The negative association between IGF-1 and FM (%) in the NMetSA group, and therefore the positive association between IGF-1 and FFM (%), is more complicated to explain since this indicates that the higher the FM, the lower the IGF-1. However, it is worth mentioning that even though neither FM (%), nor IGF-1 differed between the groups, overall, the NMetSA group displayed a lower mean IGF-1 level compared to the other groups which could have contributed to this negative association. Furthermore, this groups' small sample size (n=12) could have also played a role here by affecting the statistical power of this study.

4.6.2 Inflammation and anthropometric measurements: obesity and abdominal obesity's relationship with metabolic status and cancer risk

Adipose tissue distribution is also proposed to have a primary role in the pathophysiology of the MetS and cancer risk by affecting inflammatory mediators (Jung & Choi, 2014; Shrivastava *et al*., 2015). The MetS is characterized by a deregulated inflammatory profile, which leads to a chronic low-grade inflammatory state (den Engelsen *et al*., 2012). Supporting evidence shows that various inflammatory mediators can contribute to the overall risk of cancer development by promoting cell-growth and angiogenesis, and decreasing apoptosis (Pèrez-Hernández *et al*., 2014; Rodríguez *et al*., 2013). However, the role of CRP specifically remains unclear.

Correlation analysis between BMI, WC and CRP revealed moderate positive correlations for all respective groups (Figure 3.18), expect the NMetSG group. For BMI and CRP, only the correlation for the MetSA group was shown to be significant, whereas the correlation between WC and CRP reported significance in both the NMetSG and MetSG groups, as well as the MetSA group. These findings are supported by evidence showing that CRP levels increase with increasing BMI and WC are even higher when measured in individuals with an android body shape (Kao *et al*., 2009; Tolmay *et al*., 2012). Since the occurrence of both obesity and the MetS are characterized by adipose tissue dysfunction, the resultant chronic low-grade inflammation (measured by CRP) is not an uncommon occurrence through its IL-6 action (Braun *et al*., 2011; Choi *et al*., 2013). Adipose tissue itself are also responsible for the production of CRP (Braun *et al*., 2011), which increase with an increase in central obesity. In addition, evidence also reveal a positive association between BMI, TBF %, WC, VAT, SAT and CRP levels (da Cruz *et al*., 2013; Tolmay *et al*., 2012; Tsuriya *et al*., 2011).

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Although the correlation between CRP and BMI in the MetSG and NMetSG groups did not show any significance, the correlation between CRP and WC were however highly significant in these groups. The positive association in the MetSG groups might be explained by the fact that WC is a better predictor of abdominal obesity, and therefore the MetS according to the IDF definition (IDF, 2006). A possible explanation for the negative association in the NMetSG group could be related to the hypothesis that individuals with a gynoid body shape presents with a less metabolically active SAT depot, compared to VAT, which is more lipolytically active (Bjørndal *et al*., 2011).

4.6.3 Sex hormone parameters' relationship with obesity-related insulin dysfunction, metabolic status and cancer risk

Different components of the MetS have been shown to influence the synthesis and bioavailability of both oestrogen and androgens (Brand *et al*., 2011), of which adipose tissue distribution have been shown to be the main contributor, not only in the pathophysiology of the MetS and cancer risk, but also on sex hormonal profiles (Iyengar *et al*., 2013; Preis *et al*., 2010; Orbetzova *et al*., 2012). An increase in E2 has been found in overweight and obese post-menopausal women, as well as in individuals with the MetS, and it is proposed to be as a result of the excess adipose tissue (Momsesso *et al*., 2011; Weinberg *et al*., 2006). Braun *et al.* (2011) also suggested that E2 can be increased in obesity-associated hyperinsulinaemic individuals through a decrease in SHBG. Furthermore, hyperinsulinaemia can also cause defective and decreased oestrogen synthesis by inhibiting aromatase gene expression (Suba, 2012), which might explain the negative associations between BMI and E2, insulin and E2, as well as SHBG and insulin, in almost all groups (Figure 3.19). However, most of these associations were found to be negligible to weak. The MetSG group was the only group that showed significant correlations, a positive association between BMI and E2, and negative associations between insulin and E2, as well as between SHBG and insulin. These findings confirm the suggested explanations by Momesso *et al.* (2011), Weinberg *et al.* (2006) and Braun *et al.* (2011), which suggests that obesity (BMI) interferes with insulin production (increased production), thereby decreasing SHBG and increasing E2.

4.6.4 Relationship between ABSI and obesity, IGF-1 and certain MetS risk factors

Krakauer & Krakauer (2012) postulated that a high ABSI value represents a higher WC than expected for a given height and weight, which in turn correlates to a larger abdominal area. In other words, ABSI can adjust WC for a certain BMI and height (Cheung, 2014). Data on ABSI are still limited and controversial and requires further investigation to strengthen its use in disease conditions (Cheung, 2014).

A negative correlation between ABSI and BMI was evident in all four groups (Figure 3.20A&B). The MetSA and MetSG showed significant correlations, whereas a trend towards significance was evident in the NMetSG group. This finding is supported by Cheung (2014) who also showed a negative

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association between ABSI and BMI in women. Therefore, theoretically as BMI increases, WC also increases, and thus in turn ABSI will decrease. We further speculate that the NMetSG and NMetSA group' correlation might also have been significant if the sample size for each group was larger.

No significant differences were evident for ABSI and IGF-1 (Figure 3.20C&D). Although this relationship did not show any significance in any of the groups, this was the first study to report on this specific relationship using the current study population. We do however admit that the small sample sizes, as well as the large variability in IGF-1 levels are possible factors that could have attributed to this negligible relationship.

Although the use ABSI is thought to be a more reliable method to measure disease risk than traditional indices, ABSI has been found to weakly correlate with components of the MetS (Haghighatdoost *et al*., 2014; Malara *et al*., 2014). We have also observed this association between ABSI and TG in the MetSA group of the current study (Appendix IX), however our study showed significance. We also found a significant positive association between ABSI and LDL-c, which might be explained by gynoid body shapes' cardio-protective properties.

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CHAPTER 5: CONCLUSION

5.1 Introduction

The previous chapter included the interpretation of results found in the current study. In this chapter, the main findings of the study will be presented, together with conclusions, followed by study limitations, as well as future recommendations.

5.2 Summary and conclusions of the main findings

5.2.1 Prevalence of the MetS and MetS risk factors

The prevalence of the MetS was found to be considerably high in this female population, with the predominant risk factors being abdominal obesity, elevated blood pressure and decreased HDL-c levels. In addition, an alarming low number of the women without the MetS presented with no risk factors. Furthermore, the high prevalence of obesity (especially abdominal obesity) observed in this population of women may have exacerbated the prevalence of the MetS as well as the individual components. This high prevalence of the MetS and its individual components in these women are worrisome, since the MetS has been associated with an increased risk of developing various lifestyle diseases.

Women need to recognize this burden of obesity and its associated metabolic dysfunction, and should be motivated to make changes regarding their metabolic health, i.e. the MetS can be prevented, as well as reversed by cultural sensitive lifestyle interventions. The early detection, treatment and prevention of the MetS and its components may decrease disease burden of chronic lifestyle diseases.

5.2.2 The effects of metabolic status and body shape on: anthropometry, BIA, physiological and biochemical blood parameters

Firstly, women with the MetS and android body shape respectively, displayed a significantly exacerbated body composition profile. Thus, metabolic status and body shape alone were found to be predictors of anthropometric and BIA parameters. Our findings added value since the women in our study were predominantly obese and displayed android and gynoid body shapes, which may play a role in the pathophysiology of the MetS, and may thus be predictors of the MetS.

Secondly, women with the MetS displayed a deregulation in physiological, metabolic and sex hormone parameters. However, body shape alone was not found to be a predictor of any physiological and biochemical blood parameters. Lastly, both metabolic status and body shape could not predict any changes in the parameters linking the MetS to cancer risk. Thus, no accurate conclusion could be

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drawn linking the MetS or body shape and cancer risk with the data obtained from this study and future investigations are needed to elucidate these links.

5.2.3 The effects of metabolic status and body shape combined on: anthropometry, BIA, physiological and biochemical blood parameters

Metabolic status was found to have an effect on BM, BMI, HC, FM (%) and FFM (%) in women with gynoid body shape, and only on BMI in women with android body shape. The only effect of body shape (android) was evident in women without the MetS for WHR.

Metabolic status was found to have an effect on several MetS risk factors in the gynoid body shape groups i.e. WC, HDL-c, TG, SBP and DBP, while; metabolic status showed an effect on TG, SBP and DBP in the android body shape groups. The only effect of body shape (android) was evident in women without the MetS for WC. Both metabolic status and body shape did not show any effect on ABSI, TC, LDL-c, fasting insulin, CRP and all sex hormone parameters.

Thus, metabolic status and body shape combined showed an effect on various body composition, physiological and biochemical blood parameters, but did not show any effect on the parameters linking the MetS to the risk of cancer development.

We showed that body shape alone has the ability to predict ABSI, whereas metabolic status, and body shape and metabolic status combined did not. We also showed weak correlations for ABSI and MetS components. However, the findings on ABSI are still controversial and understudied, and more research is required to substantiate the use of this metric in relation to the MetS, body shape and cancer risk.

5.2.4 Correlations

Correlation analysis revealed that adipose tissue distribution in relation to the MetS and risk of cancer development may play a role in this complex pathophysiology, by showing significant relationships between body composition parameters and IGF-1, CRP and sex hormone parameters. However, our study design limits us to confer causality.

5.3 Advantages, limitations and future recommendations

5.3.1 Advantages

This current study has several advantages which include the following; (i) it was the first study to make use of ABSI as a metric in a South African setting in relation to metabolic status and body shape in this specific population of women, (ii) it investigated the pathophysiology of the MetS and cancer risk by including several factors (metabolic, growth and inflammatory factors, as well as sex hormone parameters) that may be involved in this pathophysiology, (iii) it included several

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measurements of adiposity, (iv) it included a comprehensive sex hormone profile, and (v) it made use of a body shape classification method namely photoscopic somatotyping which is not often used.

5.3.2 Limitations

The inclusion of only women in this current study may be seen as a limitation, but considering the fact that this study also focused on female hormones, thus this cannot be considered as a limitation.

As with many field studies, technical issues with data collection were encountered. For some volunteers, the BIA full body test revealed incorrect measurements, and even after repeating the measurements several times, the errors still remained. Possible reasons could include: the presence of prosthetic implants, the cold temperatures during the winter months, as well as the possibility that some of the electrode stickers did not stick properly to the specific skin areas of the participants. Secondly, due to the cross-sectional nature of this study, no inference to the total population or causality can be made. In addition, C-reactive protein is a non-specific marker of inflammation.

Although this study had some limitations, it also added value to what is currently known regarding the MetS and body shapes in relation to body composition, metabolic and sex hormone parameters and cancer pathophysiology. This study also added value regarding the use of the new body shape index, ABSI.

5.3.3 Future Recommendations

For future investigation we propose to include additional inflammatory markers, such as IL-6 which could have been used to add value to this specific study. In addition, to rule out any active infection as a source of inflammation, oral temperature or a full white blood cell count might be necessary. More sensitive ELISA kits for CRP and IGF-1 in future studies should also be recommended to detect lower levels of both CRP and IGF-1. In addition, as an alternative we propose the use of WHR to classify body shape/fat distribution and to classify women as insulin resistant or not. We could not establish in what menstrual phase each of the women were, and thus could not establish if E2 levels were lower, within range or higher than the normal physiological levels for each individual. As an alternative we propose to include factors that could have an effect on sex hormone levels. For example a full menstrual history i.e. uses of contraception, date of last menstrual cycle, number of cycles or irregular cycles, as well as age, menopausal status, parity, in future investigations.

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APPENDICES

Appendix I

Ethical approval from Stellenbosch University's Health Research Ethics Committee I

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Approval Notice
New Application

02-Sep-2013
Nell, Theodore TA

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

DearDoctor Theodore Nell,

The **New Application** received on **19-Apr-2013**, was reviewed by members of **Health Research Ethics Committee 1** via Minimal Risk Review procedures on **30-Aug-2013** and was approved.
Please note the following information about your approved research protocol:

Protocol Approval Period: **30-Aug-2013 -30-Aug-2014**

Please remember to use your **protocol number (N13/04/052)** on any documents or correspondence with the HREC concerning your research protocol.

Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

After Ethical Review:
Please note a template of the progress report is obtainable on www.sun.ac.za/rds and should be submitted to the Committee before the year has expired.
The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit.
Translation of the consent document to the language applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005239

The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Provincial and City of Cape Town Approval

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Helene Visser at City Health (Helene.Visser@capetown.gov.za Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

We wish you the best as you conduct your research.
For standard HREC forms and documents please visit: www.sun.ac.za/rds

If you have any questions or need further assistance, please contact the HREC office at 0219389657.

Included Documents:
DEC CV LOMBARD
IC FORM
DEC CV ESSOP
DEC CV NELL
DEC CV NIEWHOUDT
DEC CV OPPERMAN
APPLIC FORM
SYNOPSIS
DEC CV OLIVIER
CHECKLIST

PROTOCOL

Sincerely,

Franklin Weber
HREC Coordinator
Health Research Ethics Committee 1

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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

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APPENDICES

Appendix II

Informed consent document

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: 021 808 3147

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

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international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council 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, CRP and IGF-1.

  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, the*

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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.*

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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.

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Id Code: _____

Declaration by participant

By signing below, I ……..……. agree to take part in a research study entitled **(Cancer risk during urbanization: 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 participant **Signature of witness**

Declaration by investigator

I *(name)* ……..…… declare that:

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

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

…… ……

Signature of investigator **Signature of witness**

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Id Code: _____

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**

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APPENDICES

Appendix III

Global physical activity Questionnaire (GPAQ)

Id Code: _____

GPAQ

Physical Activity			
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. Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. *[Insert other examples if needed]*. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.			
Questions		**Response**	**Code**
Activity at work			
1	Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like *[carrying or lifting heavy loads, digging or construction work]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P 4*	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 ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P3 (a-b)
4	Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking *[or carrying light loads]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 NO 2 *If No, go to P 7*	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 ⌊_⌊_⌋ : ⌊_⌊_⌋ hrs mins	P6

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 place of worship. [insert other examples if needed]

7	Do you walk or use a bicycle (*pedal cycle*) for at least 10 minutes continuously to get to and from places?	Yes 1 No 2 *If No, go to P 10*	P7
8	In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously 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 ⌊_⌊_⌋ : ⌊_⌊_⌋ hrs mins	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), [insert relevant terms].

10	Do you do any vigorous-intensity sports, fitness or recreational (*leisure*) activities that cause large increases in breathing or heart rate like [*running or football,*] for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P 13*	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 activities on a typical day?	Hours : minutes ⌊_⌊_⌋ : ⌊_⌊_⌋ hrs mins	P12 (a-b)

GPAQ, Continued

Id Code: _____

Physical Activity (recreational activities) contd.			
Questions		**Response**	**Code**
13	Do you do any moderate-intensity sports, fitness or recreational (*leisure*) activities that cause a small increase in breathing or heart rate such as brisk walking, (*cycling, swimming, and volleyball*) for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	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 └┴┘ : └┴┘ hrs mins	P15 (a-b)
Sedentary behaviour			
The following question is about sitting 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, train, reading, playing cards or watching television], but do not include time spent sleeping. *[INSERT EXAMPLES] (USE SHOWCARD)*			
16	How much time do you usually spend sitting or reclining on a typical day?	Hours : minutes └┴┘ : └┴┘ hrs min s	P16 (a-b)

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APPENDICES

Appendix IV

Smoking and drinking Questionnaire

Id Code: _____

Previous smoker or drinker:	Yes:	No
If yes, how many and for how long:	How many:	How long:
Current smoker or drinker:	Yes:	No
If yes, how many and for how long:	How many:	How long:

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APPENDICES

Appendix V

Anthropometrical measurement sheet

CANSA Study: Anthropometrical Data Collection Sheet

Id Code:_____

Gender	**MALE**		**FEMALE**	
Age				
Body Mass (kg)				
Blood Pressure (mmHg)			**Heart Rate**	
Height (m)				
BMI (kg/m^2)				
Waist Circumference (cm)				
Hip Circumference (cm)				
SAD (cm)				
VAT				
Impedance (Ohms)				
Phase Angle				
SAT				
Impedance (Ohms)				
Phase Angle				
Body Shape				

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APPENDICES

Appendix VI

Bioelectrical impedance analysis (BIA) protocol-full test

Pre-test recommendations for participants

- No moderate or vigorous physical activity 12 hours prior to test.
- No consumption of food or beverages (fasted for at least 2 hours prior to test).
- No alcohol intake (12 hours prior to test).
- Void the bladder prior to test.
- The participant should not be diaphoretic (covered with sweat) or soaked in urine as the analyser measures this fluid as fat-free mass.

Pre-test instructions

- Make sure analyser battery is well charged.
- Setup of the BIA analyser at room temperature, calibrate the analyser and cables.
- Log participant variables prior to test (weight, height, age, gender and ethnicity).
- Firstly, show the participant the BIA analyser and clearly explain how the analyser works.
- Next, explain where you will place the respective electrode, and instruct the participant to remove his/her right shoe and sock. If, for some reason, the procedure must be done on the left side, then make a note of it, and subsequently (next visits) always use the left side.
- Ask participant to remove any jewellery/money/electronic devises.

Test Instructions

- Instruct participant to get on to exam table (the exam table should be comfortable and free of drafts and electrical source heaters) and lay quietly with minimal movement during the test.
- Have the participant lie on his/her back, arms by his/her sides and arms and thighs not touching.
- The specific skin areas (hand and foot) used for testing should be cleaned with 70 % v/v alcohol swab.

- **Attach the electrodes as follows:**
- Wrist: Place the electrode labelled 2 at the proximal of the wrist (pisiform prominence). Make sure the top of the electrode is toward the shoulder with the tab facing away from the body.

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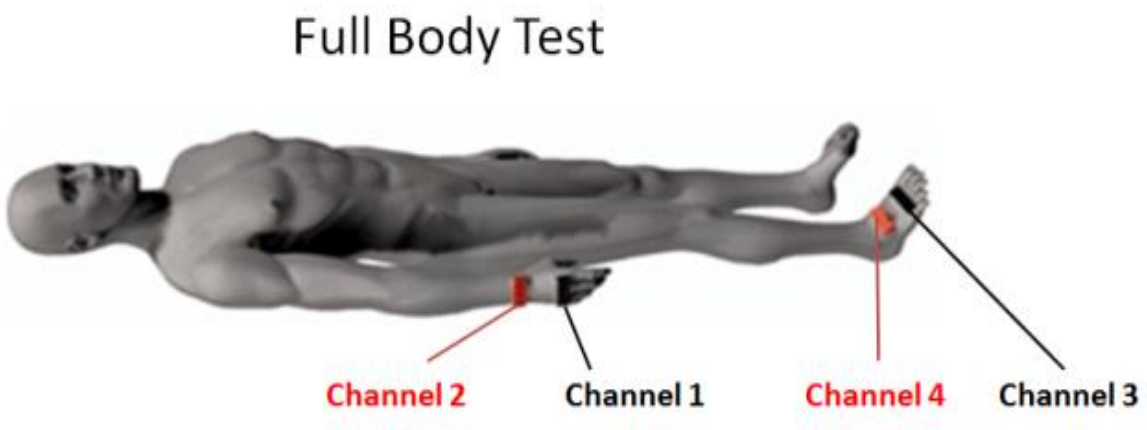
- Hand: Place the electrode labelled 1 at the dorsal surfaces of the hand (near metacarpal phalangeal joint). The tab should be facing away from the body.
- Ankle: Place the electrode labelled 4 on the ankle between medial and lateral malleoli bones. Make sure that the top of the electrode is toward the thigh with tab facing away from the body.
- Foot: Place the electrode labelled 3 at just behind the middle toes above the knuckles (near metatarsophalangeal joint).
- Attach the leads to the electrodes.
- Start test, auto save test and write down test number.



Electrode positioning for BIA full test: Channel 1: Electrode 1, Channel 2: Electrode 2, Channel 3: Electrode 3, Channel 4: Electrode 4

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APPENDICES

Appendix VII

Human C reactive protein (CRP) ELISA protocol

General considerations

Kit stored between 2-8 °C.

Reagent Preparation

All kit reagents and samples were thawed and allowed to equilibrate at room temperature (18-25 °C) prior to assay.

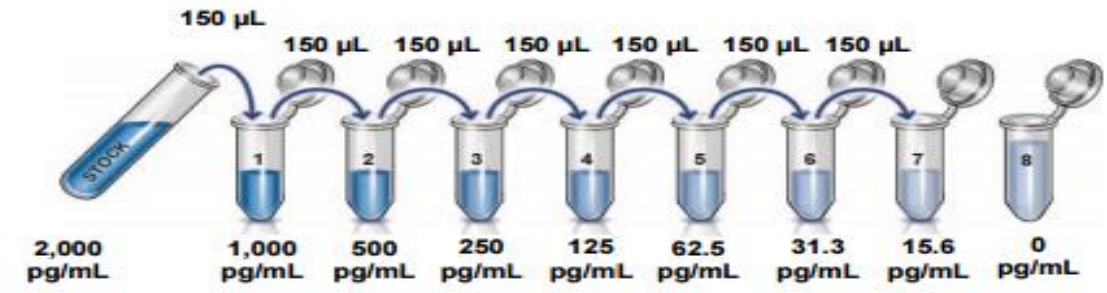
All reagents were prepared according to kit specifications (Cat ab181416).

Reagent	Preparation
1x Wash Buffer PT	Dilute 10x wash buffer with deionized water. 50 ml 1x Wash Buffer PT = 5 ml 10x wash buffer + 45 ml deionized water. Mix gently.
Antibody cocktail	Dilute the capture and detector antibodies in antibody diluent. 3ml antibody cocktail = 300 µl 10x capture antibody + 300 µl 10x detector antibody + 2.4 ml antibody diluent. Mix gently.

Standard Preparation

Prepare standards prior to use. A standard curve was constructed by an eight-serial two-fold dilution series using the CRP human lyophilized recombinant protein supplied.

1. Make up the 200 pg/ml stock standard solution. Reconstitute the CRP human protein sample by adding 200 µl of water and mix (room temperature for 5 minutes and mix again).
2. Label 8 separate tubes from standard 1-8 and add 150 µl of the sample diluent in each tube.
3. Make standards up according to figure and table below.
4. Standard 8 is blank (no protein).



Standard	Sample to dilute	Volume to dilute (µl)	Volume of diluent (µl)	Starting concentration (pg/ml)	Final concentration (pg/ml)
1	Stock	150	150	2.000	1000
2	Standard 1	150	150	1.000	500
3	Standard 2	150	150	500	250
4	Standard 3	150	150	250	125
5	Standard 4	150	150	125	62.5
6	Standard 5	150	150	62.5	31.25
7	Standard 6	150	150	31.25	15.62
8 (Blank)	None	0	150	0	0

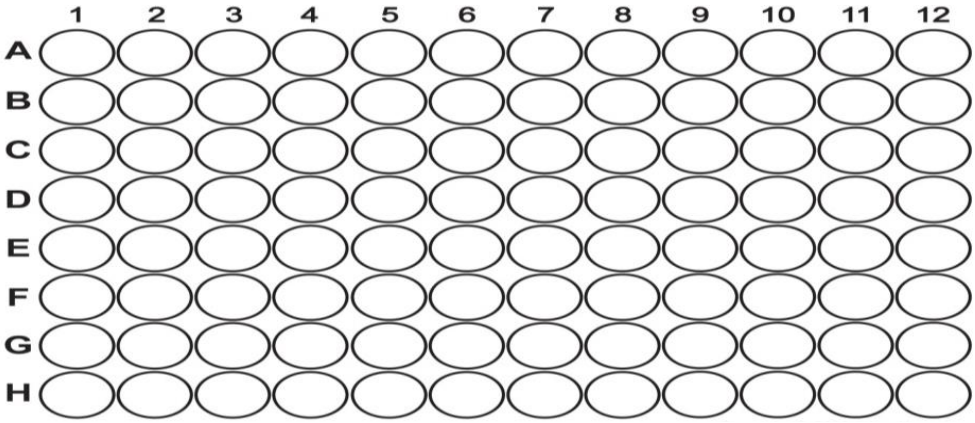
Sample Preparation

- Human serum sample dynamic dilution range: 8 000-512 000x, according to kit specifications.
- Relevant literature was consulted to estimate the serum sample range for sample population.
- Serum sample dilutions for this current study were 25 000x (see table below).
- First, dilute serum samples with 1x wash buffer and then perform final dilution with sample diluent.

Tube	Sample to diluent	Volume to diluent (µl)	Volume of 1x wash buffer (µl)	Volume of sample diluent (µl)	Starting dilution	Final dilution
1	Serum	10	240	0	neat	1:25
2	Tube 1	10	390	0	1:25	1:1000
3	Tube 2	10	0	240	1:1000	1:25 000

Microplate preparation

- Remove covering strip and add amount of plate strips needed for the assay.
- Work out the plate layout i.e. standards, blanks, positive controls and samples (prior to assay).



Assay procedure

- Prepare all reagents, standards, samples and microplate according to kit specifications.
- Add 50 µl of serum sample/control/standards in duplicate, followed by 50 µl of antibody cocktail to the assigned wells.
- Seal the micro-titre plate and incubate at room temperature for 60 minutes on a plate shaker at 400 rpm, followed by a wash step with wash buffer (three times 250 µl per well) using a plate washer.
- Invert the plate on tissue paper to remove all excess liquid.
- Add 100 µl of TMB substrate to all wells, followed by a second incubation step, in the dark on a plate shaker at 400 rpm.
- Add 100 µl of stop solution and incubate again for one minute on a plate shaker at 400 rpm.
- The optical densities of the individual wells must be measured at 450 nm on an EL800 universal microplate reader (BIO-Tek instruments, INC.) within 15 minutes after the reaction was stopped.
- Analyse data

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- The optical density is directly proportional to the CRP protein concentration of the participant sample. The concentration of all the participant samples was calculated by the formula produced by the standard curve.
- Results were obtained in pg/ml and were converted to mg/L (pg/ml/ 1 000 000= mg/L).

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APPENDICES

Appendix VIII

Human insulin-like growth factor-1 (IGF-1) ELISA protocol

General considerations

Kit stored at -20 °C.

Reagent Preparation

All kit reagents and samples were thawed and allowed to equilibrate at to room temperature (18-25 °C) prior to assay.

All reagents were prepared according to kit specifications (Cat ab100545).

Reagent	Preparation
1x wash solution	If the 20x wash concentrate contains crystals, thaw to room temperature and mix gently. 400 ml of 1x wash solution= Dilute 20 ml 20x wash buffer concentrate with deionized/ distilled water (380ml).
1x biotinylated IGF-1 detection antibody	Briefly spin down 1x biotinylated IGF-1 detection antibody vial before use. Add 100 µl of 1x assay diluent C into the vial to prepare a detection antibody concentrate (mix gently *via* pipetting). The detection antibody concentrate must be diluted 80x with 1x assay diluent prior to assay procedure. Add 100 µl of biotinylated IGF-1 detection antibody into + 8 ml assay diluent C. Mix well.
1x HRP-Streptavidin solution	Briefly spin down 120x HRP-Streptavidin concentrate vial and pipette up n down to mix before use. HRP-Streptavidin concentrate must be diluted 120x with 1x assay diluent C prior to use. 1x HRP-Streptavidin solution =Add 100 µl of 120x HRP-Streptavidin concentrate into + 12 ml assay diluent C. Mix well.

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Standard Preparation

- Prepare standards prior to use.
- Keep standard dilutions on ice while preparing.
- Do not vortex the standards as it will destabilize the protein.

A standard curve was constructed by a seven-serial two-fold dilution series, using IGF-1 human recombinant protein supplied by the kit.

1. Spin down vial very briefly before preparing standards. Make sure powder is resolved when making standards.

2. Make up the 100 ng/ml stock standard solution by adding 400 µl of 1x assay diluent C into the vial.

3. Make sure powder is resolved when making standards, by gently inverting and flicking the tube several times and flick the tube a few times. Spin down. Repeat this 3-4 times.

4. Label 6 separate tubes 1-6 and add 150 µl of the 100 ng/ml Stock standard solution to 350 µl of assay diluent in tube 1. Mix gently.

5. Add 300 µl of assay diluent C into remaining tubes.

6. Prepare standard 2 by adding 200 µl from tube 1 to tube 2 and mix.

7. Prepare standard 3 by adding 200 µl from tube 2 to tube 3 and mix.

8. Make up other standards according to figure and table below.

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Standard	Diluting Volume (µl)	Diluent (µl)	Total Volume (µl)	Starting concentration (ng/ml)	Final concentration (ng/ml)
1	150	350	500	100	30
2	200	300	500	30	12
3	200	300	500	12	4.8
4	200	300	500	4.8	1.92
5	200	300	500	1.92	0.768
6	200	300	500	0.768	0.307
7	200	300	500	0.307	0.123
8 (Blank)	0	300	300	0	0

Sample Preparation

- Serum samples should be diluted with 1x assay diluent.
- Human serum sample dynamic dilution range: 2-20x according to the kit.
- Relevant literature was consulted to estimate the serum sample range for the sample population.
- Serum samples dilution for this current study was 20x: 100 µl of serum sample per well needed, thus 200 µl as samples are tested in duplicate.
- Final sample volume is 250 µl in case of spills/pipetting errors. Thus, 12.5 µl sample plus 237.5 µl sample diluent should be used for each sample dilution.

Microplate Preparation

- Work out the plate layout, i.e. standards, blanks, positive controls and samples (prior to assay).
- Remove covering strip and add amount of plate strips needed for the assay.

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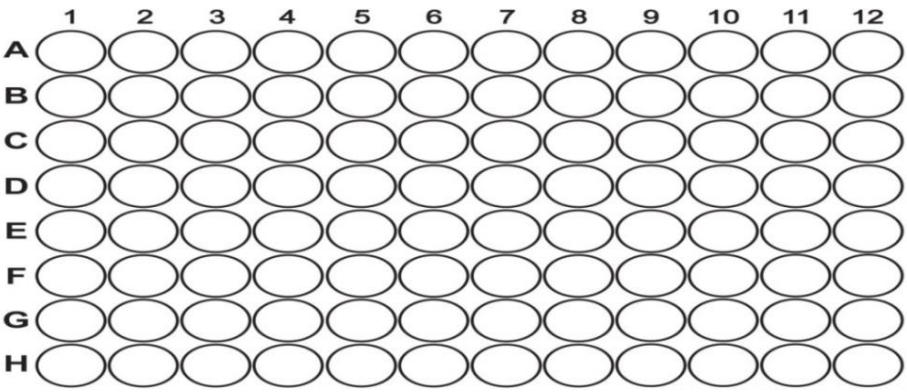

Assay procedure

- Prepare all reagents, standards, samples and microplate are prepared to kit specifications.
- Add 100 µl of each standard, serum sample or control (in duplicate) into the assigned wells, cover wells and incubate for 2.5 hours at room temperature with gentle shaking.
- Discard solution and wash 4x with 1x wash buffer solution (300 µl).
- Next invert the plate on tissue paper to remove all excess liquid.
- Add 100 µl of 1x biotinylated IGF-1 detection antibody into the assigned wells. Incubate for 60 minutes at room temperature with gentle shaking.
- Discard solution and wash 4x with 1x wash buffer solution (300 µl).
- Next invert the plate on tissue paper to remove all excess liquid.
- Add 100 µl 1x HRP-Streptavidin solution to all wells, followed by an incubation step of 45 minutes at room temperature with gentle shaking.
- Discard solution and wash 4x with 1x wash buffer solution (300 µl).
- Invert the plate on tissue paper to remove all excess liquid.
- Add 100 µl of TMB one step substrate to each well and incubate for 30 minutes at room temperature in the dark with gentle shaking.
- Add 50 µl stop solution to each well.
- The optical densities of the individual wells must be measured at 450 nm on an EL800 universal microplate reader (BIO-Tek instruments, INC. Weltevreden Park, South Africa) within 15 minutes after the reaction was stopped.
- Analyse data

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APPENDICES

Appendix IX

Additional correlations for selected biochemical and anthropometric parameters

Correlations Parameters	ABSI & TG		ABSI & LDL-c		Fasting insulin & FM %	
	r	p	r	p	r	p
MetSG	-0.045	0.87	0.31	0.24	-0.38	0.08
NMetSG	0.58	0.14	0.78	0.02	-0.29	0.19
MetSA	0.55	0.02	0.32	0.18	-0.46	0.03
NMetSA	0.59	0.13	0.12	0.77	0.09	0.79

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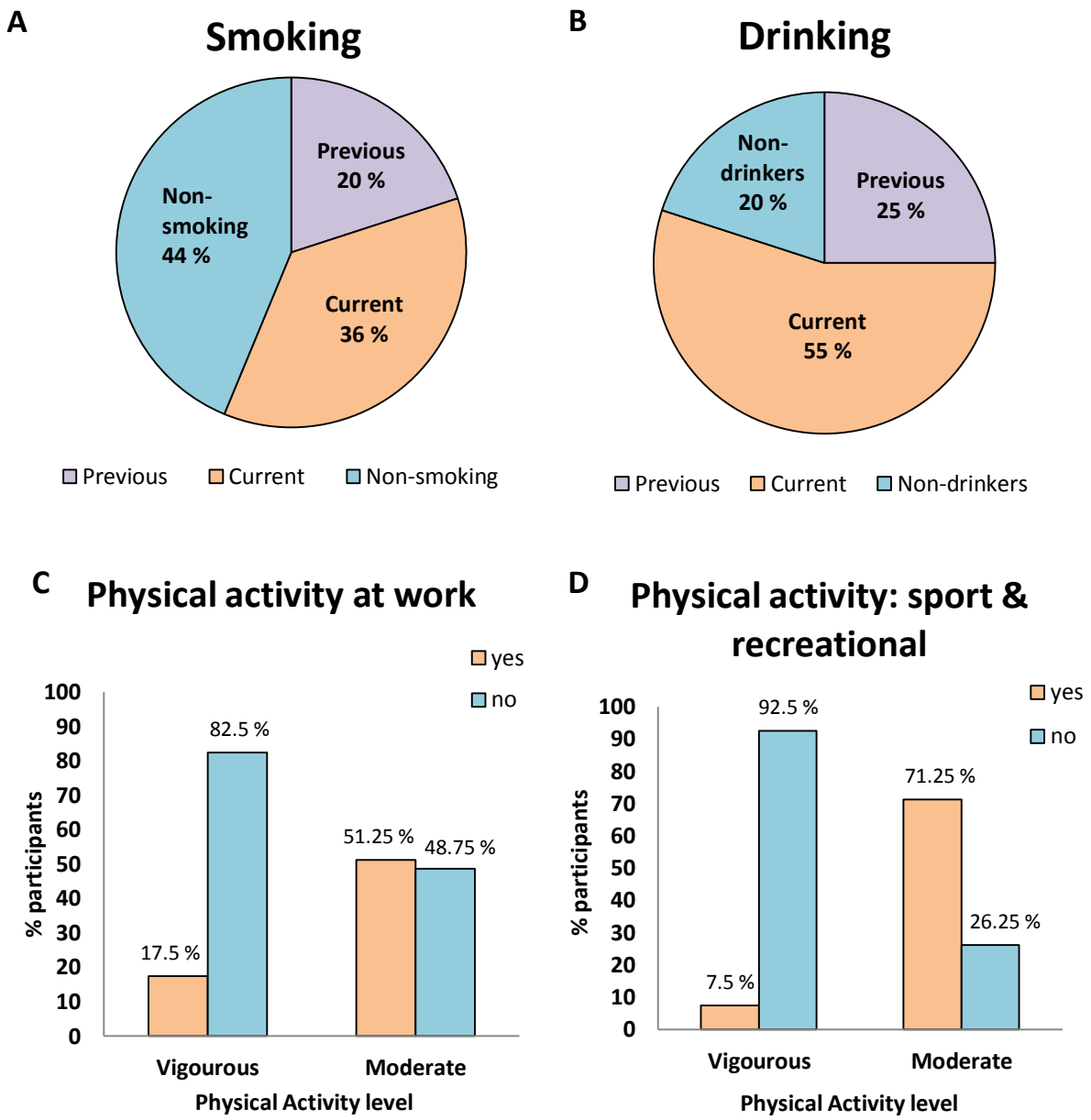
APPENDICES

Appendix X

Lifestyle related risk factors results



Lifestyle related risk factors of total population for (A) Smoking, (B) Drinking, and (C-D) Physical activity.