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THE OBESE AFRICAN WOMAN: AN ENDOCRINOLOGICAL AND CARDIOVASCULAR INVESTIGATION

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AFRIKAANSE TITEL: Die obese Afrika vrou: 'n endokrinologiese en kardiovaskulêre ondersoek

OPSOMMING

Motivering: Die voorkoms van obesiteit is die hoogste onder Afrika vroue in Suid-Afrika. Obesiteit is 'n groot kardiovaskulêre risikofaktor, en daarom kan Afrika vroue van Suid-Afrika gesien word as 'n hoë risiko groep. Ondersoeke aangaande obesiteit-verwante hipertensie is egter beperk in hierdie populasie. Die verwantskap tussen liggaamsvet verspreiding en hormone, soos leptien en endotelien-1, met kardiovaskulêre funksie is nog nie bepaal in hierdie vroue nie. Daar is gevind dat endotelien-1 'n rolspeler is in die ontwikkeling en/of instandhouding van hipertensie in verskeie populasie groepe, veral Afro-Amerikaners. Daar is ook gevind dat endotelien-1 'n rol speel in obesiteit-verwante hipertensie in nie-Afrika populasie groepe. Daar is aanduidings dat die obesiteit-verwante hormoon, leptien, ook 'n groot rol speel in obesiteit-verwante hipertensie, veral in Afro- Amerikaners, by wie gevind is dat leptienvlakke hoër is in obese hipertensiewe Afro-Amerikaanse vroue teenoor 'n obese normotensiewe kontrole groep. Aangesien hierdie twee hormone 'n prominente rol speel in obesiteit en hipertensie in Afro-Amerikaanse en nie-Afrika populasie groepe, dien die tekort aan data vir Afrika vroue van Suid-Afrika as motivering om hierdie groep te ondersoek.

Doelstelling: Om obesiteit-verwante hipertensie in Afrika-vroue te ondersoek deur assosiasies van verskeie antropometriese en endokrinologiese veranderlikes met kardiovaskulêre, veral vaskulêre funksie te bepaal.

Metodologie: In die manuskripte wat in Hoofstukke 2, 3 en 4 vervat is, word gebruik gemaak van data wat versamel is in die POWIRS (Profiles of Obese Women suffering from the Insulin Resistance Syndrome) I projek. In genoemde projek is Afrika vroue geselekteer van 'n staatsinstansie in die Noordwes Provinsie. 'n Groep van 98 vroue is verdeel in skraal normotensief, oorgewig/obese normotensief en oorgewig/obese hipertensief. Antropometriese en kardiovaskulêre metings is geneem en die lipiedprofiel, leptien en endotelien-1 vlakke is bepaal. Die kovariansie analise (ANKOVA) is gebruik om betekenisvolle verskille tussen groepe te bepaal, terwyl daar vir ouderdom gekorrigeer is. Parsiële korrelasie koëffisiënte is gebruik om assosiasies tussen veranderlikes te bepaal terwyl daar vir ouderdom gekorrigeer is. Stapsgewyse meervoudige regressie analises is ook uitgevoer om assosiasies tussen veranderlikes te bepaal. Die studie wat in Hoofstuk 5 vervat is, maak gebruik van beide POWIRS I en II, dit wil sê, studies wat beide Afrika en Kaukasiër vroue ingesluit het. Die metodologie van die twee studies is dieselfde.

Alle proefpersone het skriftelik ingeligte toestemming gegee. Die studie is goedgekeur deur die Etiekkomitee van die Noordwes-Universiteit. In die "Materials and Methods" afdeling van Hoofstuk 2-5 word die beskrywing van die proefpersone, studie-ontwerp, en analitiese metodes wat gebruik is, meer breedvoerig bespreek.

Resultate en gevolgtrekkings van die individuele manuskripte

- Die resultate van Hoofstuk 2 toon aan dat die volume-beladingseffek, wat geassosieer word met obesiteit, teenwoordig is by beide die oorgewig/obees normotensiewe en die oorgewig/obees hipertensiewe groepe. Die akkommoderingseffek wat in die oorgewig/obees normotensiewe groep verkry is, was egter afwesig in die oorgewig/obees hipertensiewe groep as gevolg van 'n afname in vaskulêre funksie. Dit was bevestig deur 'n hoë polsdruk. 'n Afname in vaskulêre funksie was geassosieer met die abdominale velvou. Dit suggereer dat abdominale subkutane, oftewel onderhuidse vet waarskynlik, óf 'n merker vir viserale vet is, óf self bydra tot 'n verhoogde kardiovaskulêre risiko in Afrika vroue.

- Resultate van Hoofstuk 3 het 'n negatiewe resultaat gelewer. Plasma endotelien-1 vlakke was dieselfde vir die skraal normotensiewe, oorgewig/obees normotensiewe en oorgewig/obees hipertensiewe groepe. Ten spyte van die herverdeling van die groepe in normotensief en hipertensief, en skraal en oorgewig/obees, is daar steeds geen verskille gevind nie. Daar is ook geen korrelasies gevind tussen endotelien-1 en kardiovaskulêre funksie in een van die groepe nie. Hierdie bevindinge suggereer dat endotelien-1 nie 'n rol speel in obesiteit-verwante hipertensie in Afrika vroue nie.

- In Hoofstuk 4 was leptienvlakke verhoog in beide die oorgewig/obese normotensiewe en hipertensiewe groepe in vergelyking met die skraal normotensiewe groep. Leptienvlakke het egter nie verskil tussen die twee oorgewig/obese groepe nie. Al was leptienvlakke dieselfde, was leptien direk en positief geassosieer met sistoliese bloeddruk en polsdruk en negatief geassosieer met arteriële meegewendheid in slegs die oorgewig/obees hipertensiewe groep, onafhanklik van obesiteit, insulienweerstand, hiperinsulienisme en ouderdom.

- In Hoofstuk 5 was die volume-belading, sowel as die akkommoderingseffek (dit is, verlaagde totale perifere weerstand en verhoogde arteriële meegewendheid) teenwoordig in beide Afrika en Kaukasiër obese groepe in vergelyking met die skraal kontrole groep. Al was leptienvlakke, liggaamsmassa-indeks en ouderdom dieselfde vir beide die Afrika en die Kaukasiër obese groepe, blyk dit dat die akkommoderingseffek

meer prominent was in die obese Kaukasiër groep, wat die laer diastoliese bloeddruk verduidelik teenoor die obese Afrika groep. Leptien toon 'n gunstige negatiewe verband met diastoliese bloeddruk en totale perifere weerstand in die obese Kaukasiër groep, maar nie in die obese Afrika groep nie. Dit mag aandui dat leptien oorwegend patologiese invloede aanwend op obese Afrika vroue, soos bepaal in Hoofstuk 4.

SLEUTELWOORDE: kardiovaskulêre funksie, obesiteit, bloeddruk, Afrika vroue, endotelien-1, leptien

SUMMARY

Motivation: The prevalence of obesity is the highest among African women in South Africa. Since obesity is a major cardiovascular risk factor, African women in South Africa could be regarded as a high risk group. However, investigations on obesity-related hypertension are limited in this population group. The associations of body fat distribution and hormones such as leptin and endothelin-1 with cardiovascular function have not yet been determined in these women. It has been determined that endothelin-1 is a role player in the development and/or maintenance of hypertension in various population groups, especially African Americans. Endothelin-1 has also been found to be involved in obesity-related hypertension in non-African population groups. It has been indicated that the obesity-related hormone, leptin, also plays a role in obesity-related hypertension, especially in African Americans. Leptin levels have been found to be higher in obese hypertensive African American women compared to an obese normotensive control group. Since the above-mentioned two hormones play a prominent role in obesity and hypertension in African American and non-African population groups, the lack of data on African women in South Africa serves as motivation to conduct this investigation.

Aim: To investigate obesity-related hypertension in African women through the determination of associations between various anthropometric and endocrinological variables with cardiovascular, especially vascular function.

Methodology: Manuscripts presented in Chapters 2, 3 and 4 made use of data from the POWIRS (Profiles of Obese Women suffering from the Insulin Resistance Syndrome) I project where African women were selected from a government institution in the North West Province. A group of 98 women were divided into lean normotensive, overweight/obese normotensive and overweight/obese hypertensive groups. Anthropometric and cardiovascular measurements were taken and the lipid profile, leptin and endothelin-1 levels determined. The analysis of covariance (ANCOVA) was used to show significant differences between groups while adjusting for age. Partial correlation coefficients were used to show associations between various variables while adjusting for age. Stepwise linear regression analysis was also used to show associations between variables. The study presented in Chapter 5 made use of both POWIRS I and II, which are studies including Africans and Caucasians, respectively. The methodology of the two studies was the same.

All subjects gave informed consent in writing and the Ethics Committee of the North-West University approved the study. The reader is referred to the "Materials and Methods" section of Chapters 2-5 for a more elaborate description of the subjects, study design and analytical methods used in each article.

Results and conclusions of the individual manuscripts

- Results from Chapter 2 showed that the volume loading effect associated with obesity was present in both overweight/obese normotensive and overweight/obese hypertensive groups, however, the accommodating effect observed in the overweight/obese normotensive group was absent in the overweight/obese hypertensive group due to decreased vascular function. This was confirmed by a high pulse pressure. Decreased vascular functioning was associated with the abdominal skinfold. This suggests that abdominal subcutaneous fat may either be a marker of visceral fat, or may in itself contribute to increased cardiovascular risk in Africans.

- Results from Chapter 3 showed a negative result. Plasma endothelin-1 levels were similar for the lean normotensive, overweight/obese normotensive and overweight/obese hypertensive groups. After re-dividing the groups into normotensive and hypertensive, and then into lean and overweight/obese, still no differences could be obtained. Additionally, no correlations could be obtained between endothelin-1 and cardiovascular function in any of the groups. These findings suggest that endothelin-1 is not implicated in obesity-related hypertension in African women.

- In Chapter 4, leptin levels were elevated in both overweight/obese normotensive and hypertensive groups compared to the lean normotensive group. However, leptin levels did not differ between the two overweight/obese groups. Even though leptin levels were the same, leptin was directly and positively associated with systolic blood pressure and pulse pressure and negatively with arterial compliance only in the overweight/obese hypertensive group, independent of obesity, insulin resistance, hyperinsulinemia and age.

- In Chapter 5 the volume loading, as well as the accommodating effect, that is, decreased total peripheral resistance and increased arterial compliance, was present in both African and Caucasian obese groups compared to their lean controls. Even though leptin levels, body mass index and age were similar for both African and Caucasian obese groups, the accommodating effect seemed to be more prominent in the obese Caucasian group, explaining a lower diastolic blood pressure compared to the obese African group. Leptin showed a favourable negative association with diastolic blood pressure and total peripheral resistance in the obese Caucasian group, but not in the obese African group. This may indicate that leptin predominantly exerts pathological influences on obese African women, as determined previously in Chapter 4.

KEY WORDS: cardiovascular function, obesity, blood pressure, African women, endothelin-1, leptin

PREFACE

For the purpose of this study it was decided to use the article format. Therefore, Chapters 2, 3, 4 and 5 are manuscripts in the form of articles. All of these articles were submitted for publication in peer reviewed journals, with Chapter 4 already being published. Although the appropriate and relevant literature backgrounds are discussed in each separate manuscript, Chapter 1 also gives an additional, more elaborate literature survey. In all of the manuscripts the promoter and co-promoters are named as co-authors. However, the main and first author initiated and was responsible for most stages of each manuscript, including literature searches, the collection of data, statistical analysis, interpretation of results and the writing of the articles. The co-authors, therefore, acted in their roles as promoter and co-promoters. All co-authors gave consent that the articles could be used in this thesis.

The first and second articles were submitted to the *Cardiovascular Journal of South Africa* and *Blood Pressure*, respectively and the third (Published) and fourth, to the *Journal of Human Hypertension*. The relevant references are provided at the end of each chapter according to the author's instructions of the specific journal in which the articles were published or submitted for publication.

AUTHOR'S CONTRIBUTIONS

The contribution of each of the researchers involved in this study is given in the following table:

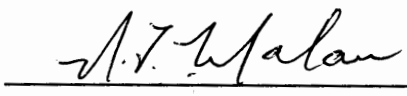
Name	Role in the study
Mr. R Schutte M.Sc. (Physiologist)	Responsible for literature searches, statistical analysis, collection of data, design and planning of manuscripts, interpretation of results and writing of all manuscripts
Dr. HW Huisman (Ph.D.) (Physiologist)	Promoter. Supervised the writing of the manuscripts, collection of data, as well as initial planning and design of manuscripts.
Dr. AE Schutte (Ph.D.) (Physiologist)	Co-promoter. Supervised the writing of the manuscripts, collection of data, as well as initial planning and design of manuscripts.
Prof. NT Malan (D.Sc.) (Physiologist)	Co-promoter. Supervised the writing of the manuscripts, collection of data, as well as initial planning and design of manuscripts.
Dr. C Underhay (Ph.D.) (Anthropometrist)	Supervised the collection of anthropometric data and gave valid scientific input in Chapter 2.

The following is a statement from the co-authors confirming their individual role in each study and giving their permission that the four manuscripts may form part of this thesis.

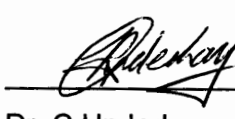
I declare that I have approved the above-mentioned manuscripts, that my role in the study, as indicated above, is representative of my actual contribution and that I hereby give my consent that they may be published as part of the Ph.D. thesis of Rudolph Schutte.

Dr. HW Huisman

Dr. AE Schutte



Prof. NT Malan



Dr. C Underhay

ABBREVIATIONS

Abdominal.	Abdominal skinfold
ANCOVA	Analysis of covariance
BMI	Body mass index
CO	Cardiac output
C_W	Arterial compliance
DBP	Diastolic blood pressure
ET_A	Endothelin-type A receptor
ET_B	Endothelin-type B receptor
ET-1	Endothelin-1
Fat %	Fat percentage
HDLC	High-density lipoprotein cholesterol
HOMA-IR	Homeostasis model assessment for insulin resistance
HR	Heart rate
Illiac crest	Illiac crest skinfold
Lean NT	Lean normotensive
LDLC	Low-density lipoprotein cholesterol
Mid axillary.	Mid axillary skinfold
n	Number of subjects
OW/OB NT	Overweight/obese normotensive
OW/OB HT	Overweight/obese hypertensive
p	Statistical significance
Pectoral.	Pectoral skinfold
POWIRS	Profiles of Obese Women with the Insulin Resistance Syndrome
PP	Pulse pressure
r	Correlation coefficient
SBP	Systolic blood pressure
Subscapular.	Subscapular skinfold
SV	Stroke volume
TC	Total cholesterol
Thigh.	Thigh skinfold
TPR	Total peripheral resistance
Triceps.	Triceps skinfold
Trig.	Triglycerides
Umbil. circum.	Umbilical circumference
Waist. circum.	Waist circumference
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

1. GENERAL INTRODUCTION

The prevalence of obesity in South Africa is the highest among African women (Anon, 1998). Although the health disadvantage associated with obesity *seems* less severe in this population group (Anon, 1998; Walker *et al.*, 2001), the heightened risk for cardiovascular disease (Shahuta *et al.*, 2004) is still present and emphasises the need to address this problem. The pathological mechanisms linking obesity to hypertension are poorly understood in general and studies on African women from South Africa are limited.

It seems that a good point of departure to investigate obesity-related hypertension in African women would be to describe the cardiovascular profile of these women with obesity-related hypertension. Since body fat distribution has been shown to be adversely associated with cardiovascular health in different population groups (Carneiro *et al.*, 2003), it would seem appropriate to attempt to draw associations between this cardiovascular profile and body fat distribution in order to determine possible adverse influences on cardiovascular function.

Endothelin-1 is believed to play a role in hypertension in African Americans (Ergul *et al.*, 1996) and also to be involved in obesity-related hypertension in Caucasians (Parrinello *et al.* 1996). Furthermore, the adipocyte-derived hormone, leptin, which is invariably elevated in the obese, is believed to be involved in obesity-related hypertension in various population groups, such as African American women (El-Gharbawy *et al.*, 2002). However, these hormones have not been investigated in African women from South Africa.

In this chapter the available literature relevant to this thesis will be discussed. However, this is a supplementary literature survey due to the appropriate and relevant literature backgrounds that are given in each separate manuscript. In the literature study an attempt will be made to describe the prevalence of obesity and hypertension in African women from South Africa. Cardiovascular function and increased adiposity, as well as the association between body fat distribution and obesity-related hypertension will be discussed. The cardiovascular, especially the vascular influences of both leptin and endothelin-1 will also be discussed. Also included in this chapter is a short motivation for each aspect (article) of this study as well as a motivation for the group subdivision. The aims and hypotheses of the studies will be stated and the structure of the thesis explained.

2. LITERATURE STUDY

2.1 THE PREVALENCE OF OBESITY AND HYPERTENSION IN AFRICAN WOMEN: THE SITUATION IN SOUTH AFRICA

In the African population of South Africa, little more than a generation ago, blood pressure and weight increments with age were not a common occurrence (Walker, 1964). However, within recent years the situation has changed considerably, especially with Africans moving to urban areas and adopting a more Westernised lifestyle (Walker *et al.*, 2001).

In South Africa, the prevalence of obesity is highest in African women and is approaching that of African American women (Walker *et al.*, 2001). According to the South African Health Review (Anon., 1998), African women have the highest average body mass index (BMI) of 27.6 kg/m^2, from which 37.7% are considered lean (18.5-24.9 kg/m^2), 25.9% overweight (25-29.9 kg/m^2) and 31.2% obese (≥ 30 kg/m^2) (WHO, 1997), followed by people of mixed origin (average BMI: 27.0 kg/m^2 – 36.1% lean; 25.3% overweight; 28.5% obese), Caucasians (average BMI: 26.5 kg/m^2 – 44.2% lean; 27.4% overweight; 25.5% obese) and Asians (average BMI: 25.1 kg/m^2 – 35.8% lean; 27.3% overweight; 21.3% obese).

No data is available at present regarding the prevalence of hypertension in each of the lean, overweight and obese subdivisions of the different ethnic groups. Since African women in South Africa have the highest average BMI, one would expect this group to have the highest prevalence of hypertension. However, this is not the case. According to the same health review (Anon., 1998), the prevalence of hypertension is highest in people of mixed origin (29.5%), followed by Caucasians (29.1%), Africans (23.5%) and Asians (22.1%). Thus, African women have the highest average BMI, but the second-lowest prevalence of hypertension. However, the heightened risk for cardiovascular disease in African women cannot be ignored.

2.2 CARDIOVASCULAR FUNCTION AND THE INFLUENCE OF INCREASED ADIPOSITY, BODY FAT DISTRIBUTION, ENDOTHELIN-1 AND LEPTIN

2.2.1 Vascular Function

2.2.1.1 The vascular system

Among the major fluid compartments, the vascular volume is the smallest, being a mere 5% of a person's total body weight. Once growth is complete, this fluid compartment remains relatively constant until old age (Sjostrand, 1949). It is also the most dynamic component, turning over completely every minute (Plante, 2002).

The endothelial layer, which paves the internal layer of the vascular system, is the cell population most exposed to physical injury, shear stress and other potentially damaging processes, even under conditions of normal blood pressure (Sarabi & Lind, 2001). Apart from their vulnerability to injury, vascular endothelial cells are uniquely equipped to deal with the stresses of ongoing fluid movement, as well as wide pressure variations (Plante, 2002, Plante, 2003). These cells are capable of producing many vasoactive agents and a variety of growth factors involved in production of basement membranes and interstitial macromolecules (Et-Taouil *et al.*, 2003). Endothelial cells further exhibit a remarkable ability to communicate with each other via gap junctions and are, therefore, able to react in a co-ordinated fashion to potentially injurious challenges (Plante *et al.*, 1996). Arterial hypertension of any type is associated with physical stress on blood vessel walls in all segments of the vasculature. Elevated pulse pressure (an increased difference between systolic and diastolic blood pressure), which results mainly from increased rigidity of large arteries, has been shown to represent an important risk factor for cardiovascular morbidity and mortality (Miura *et al.*, 2001). This elevated pulse pressure affects the smaller arteries, inducing vascular smooth muscle cell hypertrophy, which contributes to peripheral resistance and leads to established hypertension (Et-Taouil *et al.*, 2001).

2.2.1.2 Total peripheral resistance

For the human body to maintain a constant blood pressure during rest there needs to be a balance between cardiac output (CO) and total peripheral resistance (TPR). TPR is determined by resistance vessels, which are predominantly small arteries, arterioles and capillaries (Van Bortel & Spek, 1998).

The hemodynamic hallmark of hypertension is a reduction in calibre of these small resistance vessels which increases TPR and blood pressure (Middlemost, 1999). Hormonal factors such as endothelin-1 cause vasoconstriction, which increases TPR, but also cause vasodilation, which decreases TPR through its actions on different receptors namely ET_A and ET_B (Ergul, 2000). Physical factors such as increased pulse pressure due to decreased arterial compliance enhances physical stress in these resistance vessels. This causes vascular smooth muscle cells to become hypertrophic, increasing resistance to blood flow and increasing arterial pressure (Plante, 2002).

2.2.1.3 Arterial compliance

Apart from the conduit function of large arteries, large arteries also temporarily store the flow jet coming from the heart in order to obtain a more continuous tissue perfusion (Van Bortel & Spek, 1998). This storing or buffering function is reflected by the compliance of the large artery (Van Bortel & Spek, 1998). Compliance, commonly referred to as Windkessel arterial compliance (C_W), is defined as the change in volume of the artery per unit of pressure (ΔV / ΔP) (Lévy & Safar, 1990). That is, a measure of the capacity of a volume-containing structure, in this case the arterial system, to accommodate further increases in volume (Dart, 2001).

The Windkessel model describes the circulation in terms of parallel resistance and capacitance components. The resistance element corresponds to measured TPR, while the capacitance element corresponds to the C_W of the arterial circulation (Dart, 2001). While C_W is widely distributed through the arterial tree, total systemic C_W is predominantly determined by the aorta and its major branches (Kelly *et al.*, 1992).

Increased pulse pressure (PP) results from vascular stiffening, reduced C_W and distensibility of central conduit blood vessels, which increases systolic blood pressure (SBP) and tends to decrease diastolic blood pressure (DBP) (Beltran *et al.*, 2001).

SBP increases because the buffering function to accommodate the systolic volume ejected by the left ventricle cannot be performed without a significant rise in peak blood pressure (O'Rourke, 2002). Reflected waves play a critical role in this rise in peak pressure. These waves originate at different sites that have not been anatomically localised, but that may be generated as vessels progressively branch out and may occur particularly at the level of smaller resistance arteries at sites of increased impedance (Schiffrin, 2004).

When vessels are stiffer, these reflections occur earlier in the cardiac cycle as pulse wave velocity is increased (Schiffrin, 2004). They, therefore, arrive at the origin of the aorta increasingly ahead of the dicrotic notch in the aortic pulse waveform, resulting in summation with the anterograde wave and increased peak pressure (Figure 1). This amplification contributes significantly to exaggerated aortic and peripheral SBP and PP (Schiffrin, 2004).

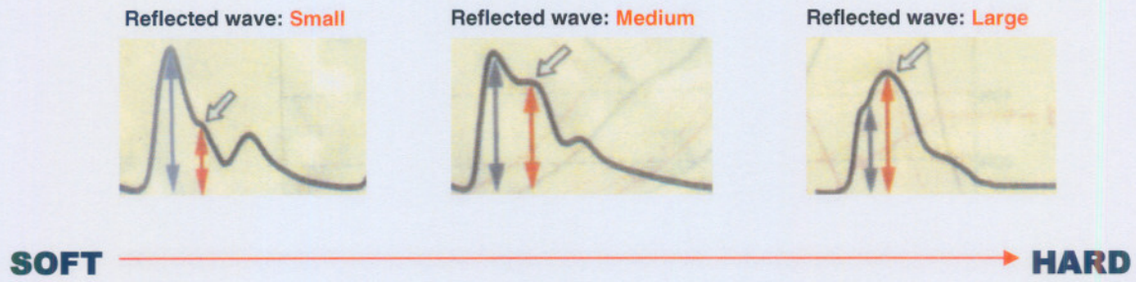


Figure 1: Reflected wave amplitude increases as the stiffness of blood vessels progresses.

2.2.2 Cardiovascular Function and Increased Adiposity

Increased adiposity has long been recognised as a significant contributor to persistently elevated blood pressure in various population groups (Must *et al.*, 1999; Stamler *et al.*, 1976; Kannel *et al.*, 1993; He *et al.*, 1994; Reed *et al.*, 1982). Although studies have consistently shown that weight gain increases blood pressure and that weight loss decreases it, the mechanisms underlying this relationship are not fully understood (Sharma, 2003).

The hemodynamic profile of obese normotensive subjects is characterised by a high intravascular volume, high CO and inappropriately normal TPR (Frolich *et al.*, 1983; Messerli *et al.*, 1981). Because heart rate (HR) remains unchanged, the increase in CO in response to the elevated metabolic requirement of adipose tissue and expanded intravascular volume occurs chiefly through increased stroke volume (SV) (Zhang & Reisin, 2000). The hemodynamic changes in obese hypertensive subjects is also characterised by an elevated CO, but TPR is also increased (Zhang & Reisin, 2000; Taler *et al.*, 2004) and C_W decreased (Wildman *et al.*, 2003), leading to increases in arterial pressure (Figure 2).

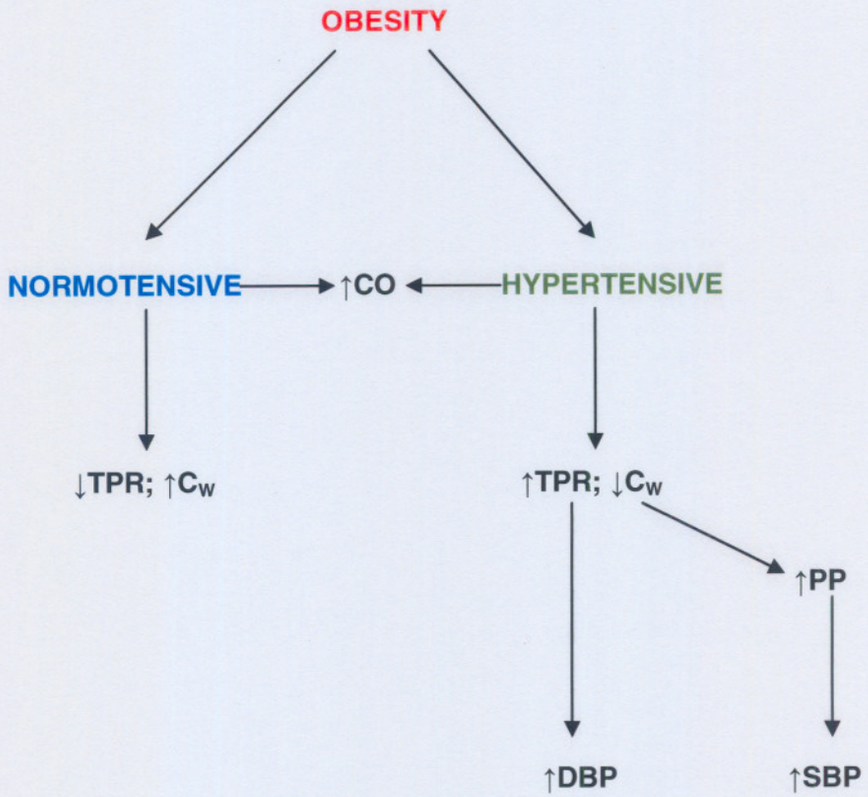


Figure 2: Cardiovascular profile of obese normotensives and hypertensives. CO (cardiac output); TPR (total peripheral resistance); C_W (arterial compliance); systolic blood pressure (SBP); diastolic blood pressure (DBP); pulse pressure (PP).

2.2.3 Body Fat Distribution and the Development of Hypertension

Body fat distribution is believed to play a role in the development of hypertension (Carneiro *et al.*, 2003). Central or abdominal obesity contributes significantly to the metabolic perturbations and cardiovascular risks in humans (Licata *et al.*, 1994; Smith *et al.*, 2001). Abdominal adipose tissue depots (visceral and subcutaneous) are metabolically active and appear to be very important in the pathogenesis of insulin resistance, dyslipidemia, glucose intolerance and hypertension (Misra & Vikram, 2003; Plavnic *et al.*, 2001).

Misra and Vikram (2003) state that BMI and body circumferences are imperfect estimates of body fat due to muscle, connective tissue, bones and body fluids that are estimated in addition to body fat. Similarly, although skinfolds provide approximate measures of subcutaneous adipose tissue in different regions of the body, they fail to provide estimates of non-subcutaneous adipose tissue (Bonora *et al.*, 1995). Nonetheless, these anthropometric measures are easy to perform and inexpensive, and roughly correlate with

the metabolic and cardiovascular endpoints associated with obesity (Misra & Vikram, 2003).

Although the above-mentioned criticism exists regarding the use of skinfolds as estimates of body fat distribution, Sardinha *et al.* (2000) determined that subcutaneous central fat, as estimated by skinfolds, is an independent predictor of cardiovascular disease risk factors, making these estimates useful, especially in epidemiological studies.

Additionally, controversy exists regarding the use of BMI, waist circumference or waist-to-hip ratio as the best determinant of obesity in population based studies. Studies by Dalton *et al.* (2003) (on Australian adults) and Pua and Ong (2005) (on Singaporean women) compared these three measures of obesity with type 2 diabetes, hypertension and dyslipidemia and concluded that these three measures of obesity performed similarly. However, these measures may perform differently in other ethnic groups and still need to be clarified (Razak *et al.*, 2005).

It would, therefore, seem more acceptable to use measures such as BMI and waist circumference in combination with subcutaneous fat distributions (determined by skinfolds) in epidemiological studies to investigate obesity and obesity-related hypertension in a population group.

2.2.4 Endothelin-1 and Vascular Function

Endothelin-1 (ET-1) is a 21 amino-acid peptide produced in many tissues (Franceschini *et al.*, 2001). Initially discovered as a product of the vascular endothelium (Yanagisawa *et al.*, 1988), this peptide has also been shown to be produced in vascular smooth muscle cells and elsewhere by other cells in different tissues (Hahn *et al.*, 1990). ET-1 is a potent and long-lasting vasoconstrictor and it is generally accepted that an increased production of ET-1 may contribute to the pathogenesis of a number of cardiovascular diseases (Nayler, 1990).

Controversy exists regarding the use of circulating ET-1 levels in epidemiological studies. ET-1 is primarily released basolaterally from the vascular endothelium to elicit smooth muscle cell contractions (Wager *et al.*, 1992) (Figure 3). Therefore, circulating (plasma) ET-1 is believed to be the result of spillover from the vascular wall and may reflect only a minor portion of total ET-1 synthesis (Treiber *et al.*, 2000). The use of circulating ET-1 still seems relevant, since higher levels would thus represent a larger spillover and an

upregulation of the endothelin system in the subject group under study. However, establishing statistical associations would seem more difficult. Nonetheless, studies do investigate circulating ET-1 levels.

Several studies have addressed the role of ET-1 in the development and maintenance of hypertension (Naruse *et al.*, 1991; Miyauchi *et al.*, 1992; Januszewicz *et al.*, 1994). Parrinello *et al.* (1996) assessed the possible role of ET-1 in the association between obesity and hypertension in Caucasians and determined that ET-1 levels were higher in obese hypertensives and obese normotensives than in lean normotensives. Additionally, ET-1 levels were also higher in obese hypertensives than in obese normotensives. Recently, Cardillo *et al.* (2004) came closer to the role of ET-1 in the pathogenesis of obesity-related hypertension in Caucasians by establishing that obese hypertensives had an enhanced endothelin-1-dependent vasoconstrictor activity.

Ergul *et al.* (1996) were the first to show racial differences in plasma ET-1 concentrations. Additionally, they found that ET-1 levels are elevated in hypertensive African Americans compared to normotensive African Americans and hypertensive Caucasians. Ergul *et al.* (1999) provided further evidence for the upregulation of the endothelin system in African Americans by indicating that African Americans possess a higher ratio of vasoconstriction-promoting endothelin-type A (ET_A) receptors in saphenous vein preparations. Furthermore, Campia *et al.* (2004) established that hypertensive African Americans have an enhanced endothelin-dependent vasoconstrictor tone compared to normotensive controls.

As mentioned, the potent vasoconstrictor and growth promoting effect of ET-1 is mediated mainly by the ET_A receptor present on vascular smooth muscle cells (Arai *et al.*, 1990) (Figure 3). However, endothelin-type B (ET_B) receptors are also present on these smooth muscle cells which also mediate these vasoconstrictive and growth promoting effects of ET-1. The vasoconstricting effect of ET-1 is increased in atherosclerotic arteries in which the vasodilatory effect of nitric oxide is impaired (Lopez *et al.*, 1990) and ET-1 acting via the ET_A receptor additionally inhibits nitric oxide synthesis (Ikeda *et al.*, 1997) and promotes vascular hypertrophy through vascular smooth muscle proliferation (Schiffrin, 1995). This would cause TPR to increase and C_W to decrease, resulting in blood pressure elevation. On the other hand, ET-1 also has vasodilatory effects by stimulating the production of nitric oxide through the activation of ET_B receptors on adjacent endothelial cells (Schiffrin, 1995) (Figure 3). In the hypertensive state, expression of the elements of the endothelin system, pre-pro endothelin-1 (PPET-1), endothelin converting enzyme

(ECE), ET_A and ET_B receptors may be upregulated in response to several factors, such as shear stress and hyperinsulinemia (Ergul, 2000) (Figure 3).

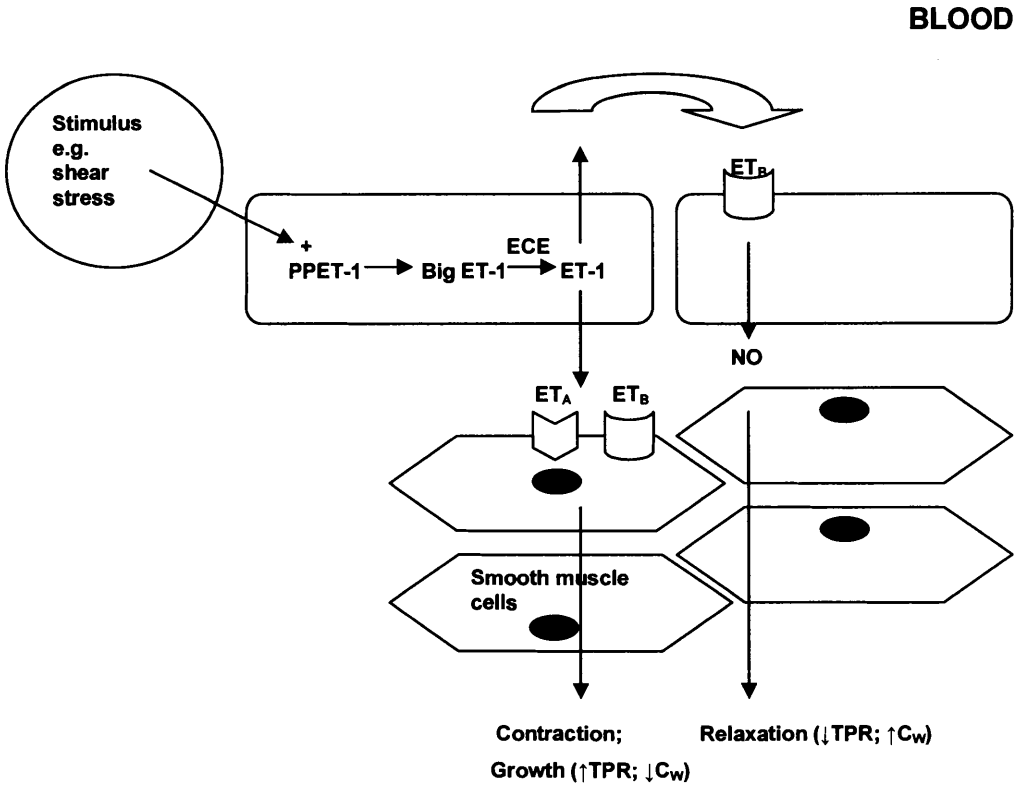


Figure 3: The dual pathways of ET-1 causing vasoconstriction, smooth muscle cell growth and vasodilation. PPET (pre-pro endothelin-1); Big ET-1 (big endothelin-1); ECE (endothelin converting enzyme); ET-1 (endothelin-1); ET_A (endothelin-type A receptor); ET_B (endothelin-type B receptor); NO (nitric oxide); TPR (total peripheral resistance); C_W (arterial compliance). Adapted from Ergul, (2000).

The above-mentioned findings have established some of the influences of ET-1 in obesity-related hypertension, without considering race, and also the influence of ET-1 in African Americans, without considering obesity, thus raising the question of the influence of ET-1 in obesity-related hypertension in Africans.

2.2.5 Leptin and Vascular Function

Leptin (from the Greek word *leptos* – meaning thin) was identified by positional cloning in 1994 (Zhang *et al.*, 1994) as a key molecule in the regulation of body weight and energy balance. It is a 167 amino acid secreted protein encoded by the *ob* gene and is predominantly expressed by white adipocytes (Maffei *et al.*, 1995) and accordingly, correlates well with body fat mass (Considine *et al.*, 1996).

Leptin crosses the blood-brain barrier through a saturable transport system and acts on receptors in the lateral and medial regions of the hypothalamus to regulate energy balance

by decreasing appetite and increasing energy expenditure through sympathetic stimulation (Hall *et al.*, 2001). Leptin levels are invariably elevated in the obese and the term "hyperleptinemia" is often referred to (Njelekela *et al.*, 2003). This condition of hyperleptinemia in the obese seems contradictory because one would expect high leptin levels to counteract obesity due to its weight reducing effects. Thus, a condition of leptin resistance prevails in the obese (Arch *et* al., 1998; Correia *et al.*, 2002). This has been observed in several murine models of obesity, including agouti obese mice, which exhibit resistance to the anorexic and weight-reducing effects of leptin (Correia *et al.*, 2002). Consequently, much attention has been focused on leptin as a possible link between obesity and hypertension in humans (El-Gharbawy *et al.*, 2002). Leptin has been associated with blood pressure in various population groups (Henriksen *et al.*, 2000; Li *et al.*, 2003; Molchanova *et al.*, 2003; Guagnano *et al.*, 2003) and has been shown to be an independent predictor of cardiovascular morbidity and mortality (Soderberg *et al.*, 1999; Wallace *et al.*, 2001; Soderberg *et al.*, 1999). Leptin levels have also been found to be higher in obese hypertensive African American women compared to obese normotensive African American women (El-Gharbawy *et al.*, 2002). Due to leptin's association with blood pressure in non-African population groups and the higher levels observed in obese hypertensive African American women compared to normotensive controls, EL-Gharbawy *et al.* (2002) attempted to obtain a direct association between leptin and blood pressure in these obese hypertensive African American women, but failed to do so.

One way in which leptin possibly increases blood pressure is by decreasing vascular function. Ciccone *et al.* (2001) found that leptin levels were independently associated with intima-media thickness of the common carotid artery, followed by Singhal *et al.* (2002), associating elevated leptin concentrations with impaired arterial distensibility. This decreased vascular function would then increase TPR and decrease C_W, resulting in blood pressure elevation. Another possible mechanism for leptin increasing blood pressure is through activation of the sympathetic nervous system (Matsumura *et al.*, 2003). One of the main consequences of this activation is to cause renal sympathetic nerve activity to increase, leading to sodium retention, volume expansion and increased blood pressure (Antic *et al.*, 2003). Additionally, angiotensin II production also increases as a consequence of this activation, increasing plasma levels and resulting in vasoconstriction and blood pressure elevation (Antic *et al.*, 2003).

On the other hand, leptin also has hypotensive effects. Frühbeck (1999) found that leptin causes vasodilation through the stimulation of nitric oxide production. This was confirmed by Beltowski *et al.* (2002), who in addition found that when inducing acute nitric oxide

blockade, leptin still prevented blood pressure elevation, suggesting that leptin also triggers additional hypotensive mechanisms. This seems possible since leptin receptors have been identified on the vascular endothelium (Sierra-Honigmann *et al.*, 1998) and smooth muscle cells (Oda *et al.*, 2001). However, in these studies, the acute effects of leptin infusion were investigated and not the effects of chronically elevated leptin levels, as observed in the obese. Leptin may also exert hypotensive effects by stimulating angiogenesis, which is the formation and organisation of new blood vessels from the pre-existing vasculature (Bouloumie *et al.*, 1998). This could lead to decreases in TPR and blood pressure because of the larger capacity.

Additionally, leptin levels have been found to be higher in African American women compared to Caucasian women (Ruhl & Everhart, 2001; Ruhl *et al.*, 2004). Generally, African American women have higher levels of subcutaneous adipose tissue compared to Caucasian women (Kanaley *et al.*, 2003). Since leptin is secreted mainly by subcutaneous white adipose tissue (Takahashi *et al.*, 1996), this possibly explains the higher leptin levels in African American women and makes leptin a strong candidate as a possible link between obesity and hypertension in African American as well as African women.

3. MOTIVATION FOR THE DIFFERENT ASPECTS OF THIS STUDY

This thesis consists of articles submitted for publication. Since the relevant literature background for each aspect is discussed in the articles, only a brief motivation for each chosen topic will be provided here.

3.1 CARDIOVASCULAR FUNCTION OF AFRICAN WOMEN WITH DIFFERENT BMI'S AND BLOOD PRESSURES

The detailed cardiovascular profiles of African women with different levels of adiposity and blood pressures have not been described. Body fat distribution, especially an abdominal distribution (Misra & Vikram, 2003) and lipid abnormalities (Kannel *et al.*, 1979; Gordon *et al.*, 1989; Hokanson & Austin, 1996) are often observed in obese hypertensives and are associated with an increased risk for cardiovascular events in various population groups (Misra & Vikram, 2003; Kannel *et al.*, 1979; Gordon *et al.*, 1989; Hokanson & Austin, 1996). However, associations between lipid abnormalities and different body fat distributions with the above-mentioned cardiovascular profiles of African women from South Africa have not been assessed.

It was, therefore, decided to compare the cardiovascular profiles of African women with different body mass indexes and blood pressures and to describe possible adverse influences to normal cardiovascular function in this group.

3.2 PLASMA ENDOTHELIN-1 IN HYPERTENSIVE AFRICAN WOMEN WITH INCREASED ADIPOSITY

ET-1 is believed to play a role in the development and maintenance of hypertension (Naruse *et al.*, 1991; Miyauchi *et al.*, 1992; Januszewicz *et al.*, 1994). It has been found to be elevated in both hypertensive African-Americans (Ergul *et al.*, 1996) (where obesity was not considered) and hypertensive obese Caucasians (Parrinello *et al.* 1996) (where differences in race were not considered) with an enhanced ET-1-dependent vasoconstrictor tone in both cases (Campia *et al.* 2004; Cardillo *et al.* 2004). ET-1 has not been investigated in Africans from South Africa. It was, therefore, decided to determine whether ET-1 levels are elevated in overweight/obese hypertensive African women compared to overweight/obese normotensive African women. Since an enhanced vasoconstrictor tone would increase TPR and decrease C_W, it was also decided to establish whether ET-1 levels are associated with an increased TPR and decreased C_W in overweight/obese hypertensive African women.

3.3 LEPTIN IS INDEPENDENTLY ASSOCIATED WITH SYSTOLIC BLOOD PRESSURE, PULSE PRESSURE AND ARTERIAL COMPLIANCE IN HYPERTENSIVE AFRICAN WOMEN WITH INCREASED ADIPOSITY: THE *POWIRS* STUDY

Leptin levels have been found to be higher in obese hypertensive African American women compared to obese normotensive African American women (El-Gharbawy *et al.*, 2002). El-Gharbawy *et al.* (2002) attempted to obtain an independent association between leptin and blood pressure in this group by adjusting for obesity, insulin resistance and hyperinsulinemia, since these three conditions are associated with both leptin and blood pressure. After these adjustments, this independent association could not be obtained. Additionally, Wildman *et al.* (2003) determined in obese African American women that excess body weight is associated with higher aortic stiffness (resulting in reduced C_W), however, leptin was not included in the study.

Since no data are available on the associations of elevated leptin levels with cardiovascular function in African women, it was decided to determine whether an independent association exists between leptin, blood pressure and C_W in

overweight/obese hypertensive African women and whether leptin levels are elevated in this group compared to overweight/obese normotensive African women.

3.4 LEPTIN IS FAVOURABLY ASSOCIATED WITH VASCULAR FUNCTION IN OBESE CAUCASIANS, BUT NOT IN OBESE AFRICANS

In obesity-related hypertension, the increased intravascular volume (Frolich *et al.*, 1983; Messerli *et al.*, 1981) cannot be adequately accommodated due to structural changes in the vascular system (Neutel *et al.*, 1999) and resulting decreased vascular function (Taler *et al.*, 2004; Wildman *et al.*, 2003). Leptin has been found to exert pressor effects through decreasing vascular function (Singhal *et al.*, 2002; Ciccone *et al.*, 2001) and increasing blood pressure (Wang *et al.*, 1999). Leptin also exerts pressor effects through increasing renal sympathetic nerve activity which results in sodium retention, volume loading and increased angiotensin II production (Matsumura *et al.*, 2003; Antic *et al.*, 2003).

On the other hand, leptin also has depressor actions by causing vasodilation after acute administration in humans (Nakagawa *et al.*, 2002).

Thus, leptin has pressor and depressor effects. Since associations of chronically elevated leptin levels with cardiovascular function in obese African women are limited (Schutte *et al.*, 2005) and comparisons of leptin's associations with cardiovascular function between Africans and Caucasians are absent, it was decided to compare leptin's associations with cardiovascular function in obese African and obese Caucasian women to determine whether leptin's associations differ between these two groups.

4. MOTIVATION FOR GROUP SUBDIVISION

BMI is often used in studies as criterion on which the subdivision of a study group is based in order to study the effects of obesity. Normally the study group is subdivided into lean, overweight and obese. Rightly so, an increase in BMI is associated with increased cardiovascular risks, such as the development of hypertension (Ginsberg, 2000). This association between excess weight and elevated blood pressure has long been recognised in African Americans (Stamler *et al.*, 1976; Kannel *et al.*, 1993; Must *et al.*, 1999), Caucasians and Asians (Reed *et al.*, 1982; He *et al.*, 1994).

Whatever the cause may be, the eventual transition from a normotensive to a hypertensive state in a person with increased adiposity is associated with structural changes in the cardiovascular system (Neutel *et al.*, 1999). Obese hypertensives fail to accommodate the volume expansion associated with increased adiposity due to an increased TPR (Taler *et al.*, 2004) and decreased C_W (Wildman *et al.*, 2003). These measures of vascular functioning are altered, in part, as a result of vascular stiffening resulting from a conglomerate of factors, such as increasing age and obesity (Schiffrin, 2004).

Evidence suggests that the health disadvantage of obesity in African women is less severe as indicated above (Anon, 1998) and seems to have little influence on their proneness to hypertension (Walker *et al.*, 2001). If this holds true, then a large number of obese normotensive subjects in a study group would statistically mask a smaller obese hypertensive group and investigations on obesity-related hypertension would prove difficult. This could be observed from a previous study on Africans, called the THUSA (Transition and Health During Urbanisation in South Africa) study which was conducted between 1996 and 1998. In brief, this was a cross sectional epidemiological study (Vorster *et al.*, 2000) which investigated the influence of urbanisation in Africans from South Africa. Various articles have been published from this study (Van Rooyen *et al.*, 2000; Huisman *et al.*, 2002; Van Rooyen *et al.*, 2002 Schutte *et al.*, 2004).

When selecting the 585 women from the above-mentioned study and dividing this group into lean (n=238), overweight (n=174) and obese (n=173), the results in Figure 4 (unpublished) became evident, depicting a "healthy" cardiovascular profile for the obese group.

Although the systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly ($p \leq 0.05$) higher in the overweight and obese groups compared to the lean groups, the SBP (117.6±1.4 mmHg) and DBP (73.8±1.0 mmHg) for the obese group were within the normotensive range of < 140/90 mmHg (Guidelines committee, 2003). From Figure 4, although the obese group's cardiac output (CO) was significantly ($p \leq 0.05$) elevated, it seems evident that the vasculature of this group shows an accommodating effect to the increased CO with the TPR decreasing and C_W increasing progressively as the measure of adiposity increases. Thus, the obese group in this case would be considered "healthy obese".

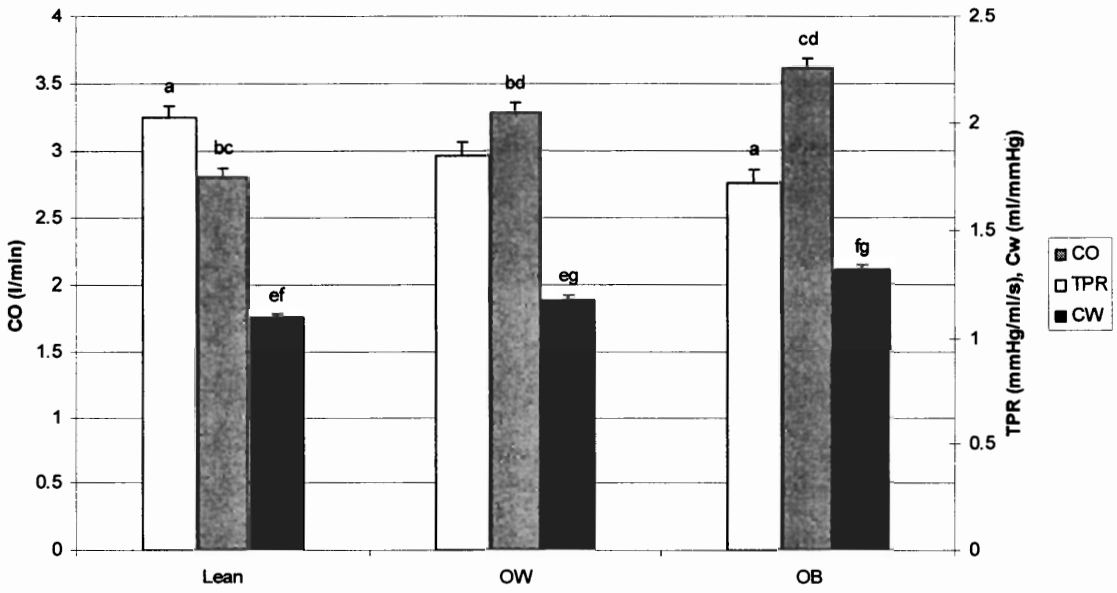


Figure 4: Comparison of lean, overweight (OW) and obese (OB) African women. Total peripheral resistance (TPR), Windkessel arterial compliance (C_W) and cardiac output (CO).

Bars with same superscript: Statistically significant ($p \leq 0.05$)
Values are age adjusted

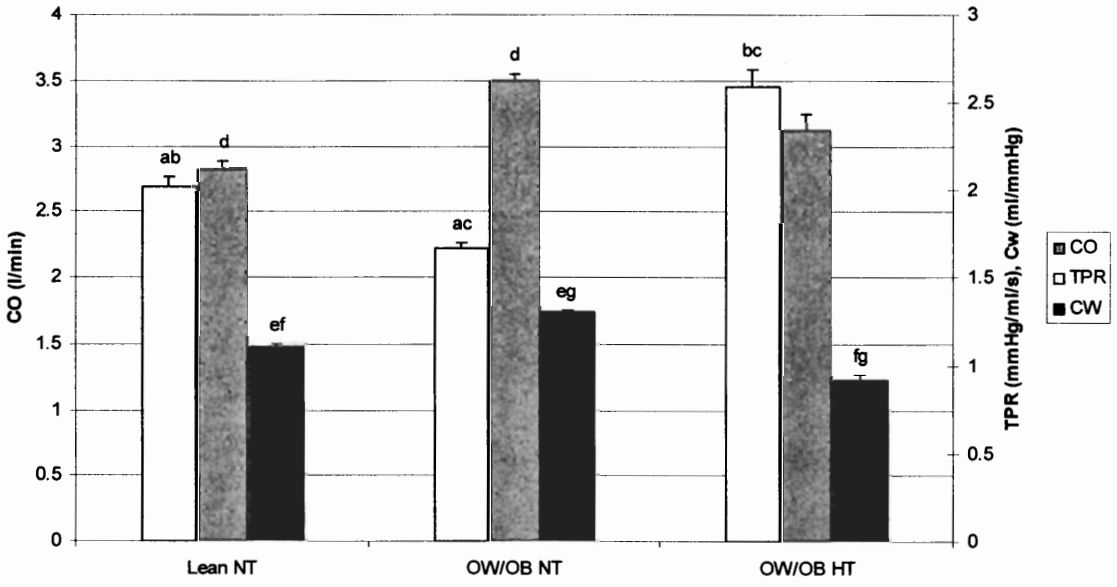


Figure 5: Comparison of lean normotensive (lean NT), overweight and obese normotensive (OW/OB NT) and overweight and obese hypertensive (OW/OB HT) African women. Total peripheral resistance (TPR), Windkessel arterial compliance (C_W) and cardiac output (CO).

Bars with same superscript: Statistically significant ($p \leq 0.05$)
Values are age adjusted

On the other hand, by adding blood pressure to the group subdivision and dividing the group into lean normotensive (lean NT), overweight/obese normotensive (OW/OB NT) and overweight/obese hypertensive (OW/OB HT), the following results in Figure 5 become

evident, highlighting the cardiovascular profile of hypertensive African women with increased adiposity.

Thus, the accommodating effect to the increased CO, as noted above in Figure 4, is still present in the OW/OB NT group, but lost in the OW/OB HT group with the TPR being significantly ($p \leq 0.05$) higher and C_W significantly ($p \leq 0.05$) lower compared to the normotensive groups.

The lean NT group consisted of 238 subjects, the OW/OB NT group of 295 subjects and the OW/OB HT group of a mere 52 subjects. This implicates that the cardiovascular profile of hypertensive African women with increased adiposity is masked when using only BMI in group subdivisions and emphasises the need to include blood pressure in order to study obesity-related hypertension in African women.

The above-mentioned group subdivisions were used in Chapters 2, 3 and 4. In Chapter 5, obesity-related hypertension, as such, was not investigated, but the comparison of leptin's associations with cardiovascular function in obese African and Caucasian women. Accordingly, only lean and obese subjects were selected.

5. AIMS

General Aim

To investigate obesity-related hypertension in African women through the determination of associations between various anthropometric and endocrinological variables with cardiovascular, and especially vascular, function.

More detailed aims of this thesis were:

Chapter 2

- To compare the cardiovascular profiles of African women with different body mass indexes and blood pressures.
- To describe possible adverse influences to normal cardiovascular function in this group of African women.

Chapter 3

- To investigate ET-1 levels in African women with different levels of adiposity and blood pressures.
- To establish whether associations exist between ET-1 and vascular function in overweight/obese hypertensive African women.

Chapter 4

- To investigate leptin levels in African women with different levels of adiposity and blood pressures.
- To determine whether leptin is directly associated with blood pressure and decreased C_W.

Chapter 5

- To compare leptin's associations with cardiovascular function in obese African and obese Caucasian women to determine whether leptin's associations differ between these two groups.

6. HYPOTHESES

The hypotheses of this thesis were:

Chapter 2

- The cardiovascular profile of overweight/obese hypertensive African women is characterised by an elevated CO, increased TPR and decreased C_W.
- The detrimental vascular profile of overweight/obese hypertensive African women is associated with a truncal fat distribution.

Chapter 3

- ET-1 levels are increased in overweight/obese hypertensive African women compared to lean normotensive and overweight/obese normotensive African women.
- ET-1 is positively associated with TPR and negatively associated with C_W in overweight/obese hypertensive African women.

Chapter 4

- Leptin levels are higher in overweight/obese hypertensive African women compared to overweight/obese normotensive African women.

- Leptin is independently associated with blood pressure and decreased C_W in overweight/obese hypertensive African women, independent of obesity, insulin resistance, hyperinsulinemia and age.

Chapter 5

- High serum leptin levels are adversely associated with vascular function in both obese African and Caucasian women.

7. STRUCTURE OF THIS THESIS

This thesis consists of four articles submitted for publication. Following this introductory chapter, Chapter 2 compares the cardiovascular profiles of normotensive and hypertensive African women with different levels of adiposity. Chapter 3 investigates plasma ET-1 levels in African women with different body mass indexes and blood pressures, while Chapter 4 determines whether an independent association exists between leptin, blood pressure and C_W in overweight/obese hypertensive African women. Chapter 5 compares leptin's associations with cardiovascular function in obese African and Caucasian women. In Chapter 6, a summary and short discussion of all the results are provided, conclusions are drawn and recommendations are made. The relevant references are provided at the end of each chapter according to the authors' instructions of the specific journal in which the articles were published or submitted for publication. The relevant references used in the unpublished Chapters 1 and 6 are provided according to the mandatory style stipulated by the North-West University.

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CHAPTER 2

CARDIOVASCULAR FUNCTION OF AFRICAN WOMEN WITH DIFFERENT BMI'S AND BLOOD PRESSURES: THE *POWIRS* STUDY

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SUMMARY

Introduction: The first aim of this study was to compare the cardiovascular profiles of a group of African women with different body mass indexes and blood pressures, and the second, to describe possible adverse influences to normal cardiovascular function in this group. **Materials and Methods:** A case-control study was performed which included 98 African women. The subjects were divided into three groups: lean normotensive (lean NT), overweight/obese normotensive (OW/OB NT) and overweight/obese hypertensive (OW/OB HT). The Finometer apparatus was used to obtain a more elaborate cardiovascular profile. The lipid profile and subcutaneous fat distributions were also determined. **Results:** A positive correlation between blood pressure and increased adiposity was obtained. Cardiac output (CO) was elevated in both OW/OB groups. Total peripheral resistance (TPR) was increased and arterial compliance (C_W) significantly decreased in the OW/OB HT group compared to the OW/OB NT group. In the total group, systolic and diastolic blood pressure could be explained best by the abdominal skinfold. The abdominal skinfold showed a direct positive association with TPR and a negative association with C_W. In the OW/OB HT group, the increased TPR could be explained best by the abdominal skinfold. **Conclusions:** In the OW/OB HT group, an increase in CO and decrease in vascular function led to the hypertensivity of this group. This seems to be related to a truncal, especially abdominal subcutaneous fat distribution. The decreased vascular function was reaffirmed by the PP exceeding 63mmHg, indicating that this group is at high risk for the development of further cardiovascular complications. Lack of significant differences between the OW/OB groups for the anthropometric and lipid profile variables and the difference in age may indicate that the younger OW/OB NT group is at high risk and should be followed up in ensuing years.

KEY WORDS: adiposity, vascular function, African women

INTRODUCTION

The prevalence of obesity is the highest among African women in South African[1] and is rapidly approaching that of African American women.[2] The heightened risk for cardiovascular disease associated with obesity[3] is a major concern in this group and emphasises the need to address this problem.

Obesity's associations with cardiovascular function in African women, as well as the associations of different body fat distributions with the cardiovascular profile have not been well described. With adipose tissue being metabolically active,[4] the metabolic and cardiovascular risks associated with obesity are closely associated with central (abdominal), rather than a peripheral (gluteo-femoral) fat pattern.[5] Much attention has been focused on the metabolic and cardiovascular risks associated with visceral abdominal adiposity,[6,7] yet the independent predictability of abdominal subcutaneous truncal fat for cardiovascular disease has also been established.[8]

Additionally, hypertension and lipid abnormalities often coexist with each being an independent risk factor for cardiovascular events.[9] The atherogenic effects of dyslipidemia have been well established,[10,11] but lipids such as triglycerides have been found to vary according to race and gender. A study by Frontini *et al.*[12] has shown that triglyceride levels were lowest in African women compared to African men and Caucasian men and women, suggesting that triglycerides may not necessarily be a significant risk factor in African women.

AIMS

The first aim of this study was to compare the cardiovascular profiles of a group of African women with different body mass indexes and blood pressures and the second, to describe possible adverse influences to normal cardiovascular function in this group.

METHODS

Participants

This study included an availability sample of 102 apparently healthy African women from a government institution in the North West Province, South Africa. Subjects were initially recruited according to the three levels of body mass index (BMI) into lean (BMI = 18.5 – 24.9 kg/m^2), overweight (BMI = 25 – 29.9) and obese (≥ 30 kg/m^2).[13] Exclusion criteria were pregnancy, lactation, diabetes mellitus and treatment for mental or neurological diseases. The mean age of this group was 31.3 ± 8.6 years. Out of the total sample of 102 subjects, three new groups were selected: Firstly, a group of 35 lean normotensive subjects, secondly, a group of 46 normotensive subjects, which consisted of a combination of overweight (n=19) and obese

(n=27) subjects and thirdly, a group of 17 newly diagnosed hypertensive subjects, also consisting of a combination of overweight (n=6) and obese (n=11) subjects. The subjects were regarded as hypertensive if their blood pressure was ≥ 140 and/or ≥ 90 mmHg.[14] A total of 98 subjects were included in the analysis of this article. The four subjects that were excluded were the only lean hypertensive subjects in the total subject group, rendering the group too small for statistical use.

The reason for this group subdivision is that a problem seems to arise when the division (into lean, overweight and obese) is based solely on BMI. Dividing the overweight/obese subjects into a normotensive and hypertensive group may enable one to indicate the detrimental cardiovascular profile of the hypertensive overweight/obese subject better that otherwise could be masked due to the large amount of normotensive subjects.

All research subjects gave informed consent in writing. The Ethics Committee of the North-West University approved the study.

Experimental Procedure

During the course of the study, the subjects reported at a metabolic ward facility (consisting of ten single bedrooms, a living room and kitchen) at 18h00 in the evening. They were all introduced to the experimental set-up including the Finometer apparatus. The purpose of the introduction to the experimental set-up was to minimise anticipation stress. The subjects received a light meal at around 19h00, which excluded caffeine and alcohol, and went to sleep before 23h00. The purpose of the dietary restrictions was to enable a good night's sleep and a stable, resting blood pressure the next morning. Finometer recordings were obtained between 06h00 and 08h00 the next morning, after the overnight rest and before breakfast. Subjects were not permitted to walk around or have anything to eat or drink (except water) until all recordings were completed.

The subject was awake and lying in the Fowler's position in a quiet single bedroom while the Finometer device was connected to the subject. Blood pressure was recorded continuously for a period of at least seven minutes. After a recording of at least two minutes, the Finometer performed a return-to-flow systolic calibration. This is an individual patient level adjustment which calibrates the upper arm pressure of each specific subject with the finger pressure. Highest precision in blood pressure readings is obtained only after this calibration. From the seven minute continuous blood pressure recording of the Finometer, the average systolic and diastolic blood pressures were determined from the last two minutes of the recording.

The Finometer device computed all cardiovascular variables online and stored the data in result files on a hard disk. The systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), heart rate (HR), stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR) and arterial compliance (C_W)[15,16] were computed and stored.

The vascular unloading technique of Peñáz together with the Physiocal criteria of Wesseling provided reliable, non-invasive and continuous estimates of blood pressure which are useable especially in comparative studies.[17,18] Since the pressure waveform is available continuously, computations provide further information on the dynamics of the cardiovascular system, similar to intra-arterial measurements.[16,19-23]

Body mass and height measurements were also taken. These anthropometric measurements were performed according to standard methods as described by Norton and Olds.[24] Maximum height was measured to the nearest 0.1 cm, with the head in the Frankfort plane, by means of a stadiometer (Invicta, IP 1465, UK). Body mass was measured to the nearest 0.1 kg by means of a calibrated electronic scale (Precision Health Scale, A&D Company, Japan). During these measurements the subjects had to stand erect with the feet together and without volitionally contracting the gluteal muscles.

The skinfolds were measured by registered anthropometrists using a Harpenden skinfold caliper with a constant pressure of $10g/m^2$. All skinfolds (triceps, subscapular, illiac crest, abdominal, pectoral, mid axillary and thigh) were measured according to the technique described in Norton *et al.*[25] and the percentage body fat was calculated, using the equation of Jackson & Pollock.[26]

After the Finometer recordings were taken, fasting blood samples were drawn from the vena cephalica or medial cubital vein and plasma and serum were prepared according to standard methods. The serum lipids were determined on a Vitros DT60 II Chemistry System with Vitros DT slides. The coefficients of variation for the lipid profile were as follows: TC – high control (2.94%), low control (0.67%); Trig. – high control (0.75%), low control (1.54%), HDLC – high control (4.87%); low control (1.61%).

Statistical analysis

The computer software package Statistica v/6.0 was used for the analysis of the data. The analysis of covariance (ANCOVA) was used to show significant differences between groups while adjusting for age. The results were seen as statistically significant when the p-value was less or equal to 0.05, which means that if the Bonferroni-adjusted confidence intervals do not overlap, the results were seen as statistically significant. Measured components were normally

distributed. Partial correlation coefficients were used to show associations between various variables while adjusting for age. Stepwise linear regression analysis using the forward stepwise method was also used to assess associations between SBP, DBP, C_W and TPR as dependent variables and some anthropometric variables as independent variables.

RESULTS

Means, standard errors and confidence intervals of cardiovascular and anthropometric variables as well as the lipid profiles are presented in Table 1.

Table 1: Means ± standard error of cardiovascular variables, anthropometric measurements and lipid profile corrected for age at a 98.3% confidence interval.

	Lean NT (n=35)	OW/OB NT (n=46)	OW/OB HT (n=17)
Age (years)	28.3±1.4 [25.7-30.9]	31.3±1.2 [29.1-33.6]	37.4±2.0 [33.7-41.1]
SBP (mmHg)	123±2.3 [118-129]^{a}	124±1.9 [120-129]^{b}	156±1.9 [148-164]^{ab}
DBP (mmHg)	72±1.4 [69-76]^{cd}	77±1.2 [74-79]^{c}	91±2.1 [86-96]^{d}
HR (bts/min)	64.8±1.6 [60.8-68.7]	70.5±1.4 [67.1-73.8]	69.1±2.4 [63.3-74.9]
SV (ml)	80.0±2.4 [74.2-85.9]	87.7±2.1 [82.7-92.7]	89.1±3.5 [80.5-97.7]
CO (l/min)	5.13±0.2 [4.67-5.59]^{ef}	6.10±0.2 [5.71-6.49]^{e}	6.09±0.3 [5.42-6.77]^{f}
TPR (mmHg/ml/s)	1.16±0.04 [1.06-1.25]	0.98±0.04 [0.90-1.06]	1.23±0.06 [1.09-1.37]
C_W(ml/mmHg)	1.78±0.04 [1.68-1.89]^{g}	2.00±0.04 [1.91-2.09]^{gh}	1.65±0.06 [1.49-1.80]^{h}
PP (mmHg)	51.2±1.8 [46.9-55.5]^{i}	47.8±1.5 [44.1-51.4]^{j}	64.7±2.6 [58.4-71.0]^{ij}
BMI (kg/m^2)	22.3±0.7 [20.5-24.0]^{kl}	31.3±0.6 [29.8-32.8]^{k}	32.4±1.1 [29.9-35.0]^{l}
Weight (kg)	57.2±1.99 [52.3-62.0]^{mn}	78.8±1.69 [74.7-82.9]^{m}	79.8±2.91 [72.7-86.9]^{n}
Triceps (mm)	15.8±1.3 [12.6-19.0]^{op}	26.4±1.1 [23.7-29.8]^{o}	30.7±1.9 [26.0-36.3]^{p}
Subscapular (mm)	12.7±1.7 [8.6-16.8]^{qr}	28.1±1.4 [24.6-31.7]^{q}	34.0±2.5 [27.9-40.0]^{r}
Illiac crest (mm)	13.7±1.5 [10.1-17.3]st	26.7±1.3 [23.7-29.8]^{s}	30.1±2.2 [24.8-35.4]^{t}
Abdominal (mm)	16.0±1.5 [12.4-19.6]^{uv}	28.4±1.3 [25.3-31.5]^{u}	34.1±2.2 [28.8-39.4]^{v}
Pectoral (mm)	7.85±1.4 [4.35-11.4]^{wx}	16.0±1.2 [13.0-19.0]^{w}	19.7±2.1 [14.5-24.8]^{x}
Mid axillary (mm)	9.79±1.4 [6.30-13.3]^{yz}	22.6±1.2 [19.6-25.6]^{y}	25.3±2.1 [20.2-30.4]^{z}
Thigh (mm)	26.4±1.4 [23.0-29.8]^{ab}	40.5±1.2 [37.6-43.4]^{a}	44.9±2.0 [39.9-49.8]^{b}
Umbil. circum. (mm)	76.5±1.9 [71.9-81.0]^{ab}	93.7±1.6 [89.8-97.5]^{a}	94.2±2.7 [87.49-100.8]^{b}
Waist circum. (mm)	96.4±1.6 [92.6-100]^{ab}	112±1.3 [109-116]^{a}	114±2.3 [109-120]^{b}
Fat %	23.4±1.3 [20.4-26.5]^{cd}	39.4±1.1 [36.8-41.1]^{c}	41.2±1.8 [36.7-45.7]^{d}
TC (mmol/l)	4.19±0.16 [3.81-4.57]	4.24±0.13 [3.91-4.56]	4.43±0.23 [3.87-4.99]
HDLC (mmol/l)	1.30±0.06 [1.16-1.44]	1.26±0.05 [1.14-1.38]	1.10±0.08 [0.89-1.30]
LDLC (mmol/l)	2.78±0.15 [2.42-3.13]	2.83±0.13 [2.52-3.13]	3.14±0.22 [2.62-3.67]
Trig. (mmol/l)	0.57±0.07 [0.41-0.73]^{e}	0.73±0.06 [0.59-0.87]	0.97±0.10 [0.73-1.21]^{e}

Lean NT (lean normotensive); OW/OB NT (overweight/obese normotensive); OW/OB HT (overweight/obese hypertensive); SBP (systolic blood pressure); DBP (diastolic blood pressure); HR (heart rate); SV (stroke volume); CO (cardiac output); TPR (total peripheral resistance); C_W (Windkessel arterial compliance); PP (pulse pressure); BMI (body mass index); Triceps (triceps skinfold); Subscapular (subscapular skinfold); Illiac crest (iliac crest skinfold); Abdominal (abdominal skinfold); Pectoral (pectoral skinfold); Mid axillary (mid axillary skinfold); Thigh (thigh skinfold); Umbil. Circum (umbilical circumference); Waist circum. (waist circumference); Fat % (fat percentage); TC (total cholesterol); HDLC (high density lipoprotein cholesterol); LDLC (low density lipoprotein cholesterol); Trig. (triglycerides)

Means with the same superscript: Statistically significant ($p \leq 0.05$)
n = 98

The groups were divided on the grounds of BMI and blood pressure and accordingly, BMI was significantly ($p \leq 0.05$) higher in the overweight and obese normotensive group (OW/OB NT) and overweight and obese hypertensive group (OW/OB HT) compared to the lean normotensive

group (lean NT). The BMI of the OW/OB NT and OW/OB HT groups were similar. The OW/OB NT group was younger ($p \leq 0.07$) than the OW/OB HT group.

CO was significantly elevated ($p \leq 0.05$) in the OW/OB groups compared to the lean NT group (Table 1; Figure 1). There was no significant difference between the OW/OB groups for CO. In the OW/OB NT group, TPR was lower ($p \leq 0.08$) and C_W significantly ($p \leq 0.05$) higher compared to the lean NT group (Table 1; Figure 1). Conversely, in the OW/OB HT group, TPR was higher ($p \leq 0.08$) and C_W significantly ($p \leq 0.05$) lower, when compared to the OW/OB NT group (Table 1; Figure 1). PP was significantly ($p \leq 0.05$) elevated in the OW/OB HT group compared to the lean NT and OW/OB NT groups.

The anthropometric variables were significantly ($p \leq 0.05$) higher in the OW/OB groups compared to the lean NT group. Although the anthropometric variables did not differ significantly between the OW/OB NT and OW/OB HT groups, the OW/OB HT group showed a tendency towards higher values (Table 1).

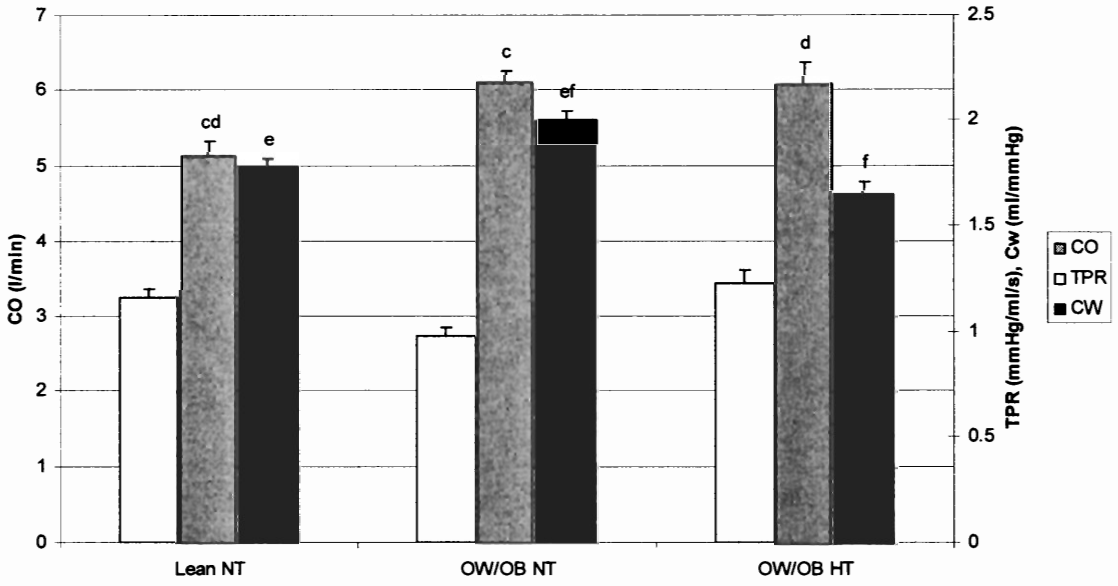

Figure 1: Comparison of lean normotensive (lean NT), overweight and obese normotensive (OW/OB NT) and overweight and obese hypertensive (OW/OB HT) African women. Total peripheral resistance (TPR), Windkessel arterial compliance (C_W) and cardiac output (CO).

Bars with same superscript: Statistically significant ($p \leq 0.05$)
Values are age adjusted

Partial correlation coefficients adjusted for age for the total group are presented in Table 2. BMI correlated positively with DBP ($p \leq 0.01$; $r=0.40$) and SBP ($p \leq 0.05$; $r=0.25$). DBP and SBP were significantly correlated with most of the anthropometric variables, indicating the positive association between adiposity and blood pressure. TPR correlated negatively with the anthropometric variables, except with the abdominal and pectoral skinfolds, while C_W correlated positively with all these variables. After additionally adjusting for BMI and waist circumference in

the total group (data not shown), a positive correlation was shown between the abdominal skinfold and TPR ($p \leq 0.05$; r=0.26) as well as a negative correlation with C_W ($p \leq 0.05$; r=-0.24).

Table 2: Partial correlation coefficients between SBP, DBP, TPR, C_W, and anthropometric variables adjusted for age

	TOTAL GROUP			
	SBP	**DBP**	**TPR**	**C_W**
BMI	$p \leq 0.05$; r=0.25	$p \leq 0.01$; r=0.40	$p \leq 0.01$; r=-0.30	$p \leq 0.01$; r=0.43
WC	$p \leq 0.05$; r=0.26	$p \leq 0.01$; r=0.34	$p \leq 0.01$; r=-0.39	$p \leq 0.01$; r=0.48
Triceps	$p \leq 0.01$; r=0.31	$p \leq 0.01$; r=0.36	$p \leq 0.01$; r=-0.28	$p \leq 0.01$; r=0.33
Subscapular	$p \leq 0.05$; r=0.25	$p \leq 0.01$; r=0.44	$p \leq 0.05$; r=-0.25	$p \leq 0.01$; r=0.28
Illiac crest	$p \leq 0.01$; r=0.29	$p \leq 0.01$; r=0.43	$p \leq 0.05$; r=-0.22	$p \leq 0.01$; r=0.27
Abdominal	$p \leq 0.05$; r=0.24	$p \leq 0.01$; r=0.44	NS	$p \leq 0.05$; r=0.23
Pectoral	NS	$p \leq 0.01$; r=0.29	NS	$p \leq 0.01$; r=0.31
Mid axillary	$p \leq 0.05$; r=0.22	$p \leq 0.01$; r=0.39	$p \leq 0.05$; r=-0.26	$p \leq 0.01$; r=0.37
Thigh	$p \leq 0.05$; r=0.26	$p \leq 0.01$; r=0.32	$p \leq 0.05$; r=-0.23	$p \leq 0.01$; r=0.29
Umbilical circum.	NS	$p \leq 0.01$; r=0.36	$p \leq 0.01$; r=-0.36	$p \leq 0.01$; r=0.48
Fat%	$p \leq 0.05$; r=0.23	$p \leq 0.01$; r=0.42	$p \leq 0.05$; r=-0.23	$p \leq 0.01$; r=0.34
HDLC	NS	NS	NS	NS
LDLC	$p \leq 0.05$; r=0.22	NS	$p \leq 0.05$; r=0.21	NS
Triglycerides	$p \leq 0.05$; r=0.21	$p \leq 0.01$; r=0.27	NS	NS

Total group (n=98); OW/OB NT (overweight/obese normotensive); OW/OB HT (overweight/obese hypertensive); SBP (systolic blood pressure); DBP (diastolic blood pressure); TPR (total peripheral resistance); C_W (Windkessel arterial compliance); BMI (body mass index); WC (waist circumference); Triceps (triceps skinfold); Subscapular (subscapular skinfold); Illiac crest (iliac crest skinfold); Abdominal (abdominal skinfold); Pectoral (pectoral skinfold); Mid axillary (mid axillary skinfold); Thigh (thigh skinfold); Umbil. Circum (umbilical circumference); Waist circum. (waist circumference); Fat % (fat percentage); TC (total cholesterol); HDLC (high density lipoprotein cholesterol); LDLC (low density lipoprotein cholesterol); TG (triglycerides)

NS = non-significant

Table 3: Stepwise linear regression analysis partial and cumulative R^2 values

Independent Variables	**Dependent variables:**		
	DBP (Total group)		
	Partial R^2	**Beta**	**Cumulative R^2**
Abdominal skinfold	*0.289*	*0.497*	*0.289*
Pectoral skinfold	0.010	-0.266	0.299
Subscapular skinfold	0.021	0.295	0.320
	SBP (Total group)		
	Partial R^2	**Beta**	**Cumulative R^2**
Abdominal skinfold	*0.187*	*0.528*	*0.187*
Triceps skinfold	0.025	0.414	0.212
Pectoral skinfold	0.021	-0.245	0.233
Umbilical circumference	0.018	-0.257	0.251
	TPR (OW/OB HT)		
	Partial R^2	**Beta**	**Cumulative R^2**
Subscapular skinfold	0.130	-0.629	0.130
Abdominal skinfold	*0.239*	*0.956*	*0.369*
Umbilical circumference	0.139	-0.567	0.508

Total group (n=98); OW/OB HT (overweight/obese hypertensive); SBP (systolic blood pressure); DBP (diastolic blood pressure); TPR (total peripheral resistance)

Independent variable in *italic*: best contributor to R^2

Using a stepwise linear regression analysis in the total group (Table 3), with DBP as dependent variable and triceps, subscapular, illiac crest, abdominal, pectoral, mid axillary and thigh skinfolds, as well as waist and umbilical circumferences as independent variables, DBP could be explained best by the abdominal skinfold. Together with pectoral and subscapular skinfolds, these three variables accounted for 32.0% of the variance in DBP (R^2=0.320). Similarly, in the same multiple regression model, SBP could also be explained best by the abdominal skinfold and together with the triceps skinfold, pectoral skinfold and umbilical circumference accounted for 25.1% of the variance in SBP (R^2=0.251) (Table 3).

In accordance with this trend observed in the total group, the increased TPR in the OW/OB HT group was explained best by the abdominal skinfold and together with the subscapular skinfold and umbilical circumference, accounted for 50.8% of the variance in TPR (R^2=0.508) (Table 3).

In the total group (Table 2), triglyceride levels showed a weak but significant correlation with SBP ($p \leq 0.05$; r=0.21) and DBP ($p \leq 0.05$; r=0.27). LDLC correlated positively with SBP ($p \leq 0.05$; r=0.22) and TPR ($p \leq 0.05$; r=0.21).

DISCUSSION

It is well documented that blood pressure rises with increasing BMI due to volume expansion and increasing CO.[27-29] Accordingly, CO was similarly elevated in both OW/OB groups in relation to the lean NT group, yet one OW/OB group was normotensive and the other hypertensive. The peripheral vasculature of the OW/OB NT group showed an accommodating effect to the raised CO by a reduced TPR and increased C_W which is in accordance with the findings of Frolich *et al.*[28] and Messerli *et al.*[29] In the OW/OB HT group, TPR failed to fall adequately and along with the decreased C_W in relation to the increased CO, contributed to the increment in blood pressure[30].

PP, which is a good determinant of aortic stiffness,[31] was significantly elevated in the OW/OB HT group compared to both the OW/OB NT and lean NT groups, indicating that a large contributing factor to the decreased C_W is due to increased aortic stiffness in the OW/OB HT group. Wildman *et al.*[32] found that excess body weight was associated with higher aortic stiffness in subjects as young as 20 to 30 years. Smulyan and Safar[31] stated further that a raised PP normally increases SBP, but decreases DBP. This is contradicted by the OW/OB HT group, with the DBP being significantly higher in the OW/OB HT group compared to the normotensive groups, even though the PP was significantly higher in this hypertensive group. Along with the increased TPR, this possibly indicates an alteration in the microcirculation as well. Studies have found PP to be independently related to cardiac hypertrophy[33] and

myocardial infarction.[34] These findings were confirmed by Fang *et al.*[35] who determined that a wide PP (>63 mmHg) is associated with subsequent cardiovascular complications. The PP of the OW/OB HT group exceeded this limit (64.7±2.6 mmHg), suggesting that this group is possibly at high risk for future cardiovascular complications.

Thus, a deterioration in micro and macrovascular functioning has been indicated in the OW/OB HT group, so a question to be asked is whether associations exist between decreased vascular functioning and the anthropometric profile in this group.

In the total group, the positive association between blood pressure and increased adiposity was confirmed. Using a stepwise linear regression analysis, both SBP and DBP could be explained best by the abdominal skinfold, indicating that this association between blood pressure and increased adiposity lies more towards the truncal area. Protsky *et al.*[36] determined that adverse changes in vascular function occur even in young normotensives (18-30 years) with an increased abdominal fat distribution. Additionally, Sardinha *et al.*[8] found that a subcutaneous truncal fat distribution is an independent predictor of cardiovascular disease. However, in the total group, increased adiposity correlated with a more healthy vascular functioning. For instance, increased adiposity correlated with a decrease in TPR and an increase in C_W, depicting an illusive healthy profile for the whole group. This is probably due to the low percentage of OW/OB HT subjects (17.3%) in this group. In an attempt to draw a direct association between vascular function and subcutaneous fat distributions, the data was additionally adjusted for BMI and waist circumference. After this adjustment in the total group, the opposite results were found, namely, the abdominal skinfold showed a weak but significant direct positive correlation with TPR as well as a negative correlation with C_W. In addition, in the OW/OB HT group, the increased TPR could be best explained by the abdominal skinfold along with the subscapular skinfold and umbilical circumference, supporting the findings by Protsky *et al.*[36] and Sardinha *et al.*[8]

The truncal skinfolds, especially the abdominal skinfold, seem to be the area of fat distribution that is associated with increased blood pressure and decreased vascular function in this group of African women. Though much emphasis has been placed on visceral fat in Eastern and Caucasian populations,[6,7] these results also show that abdominal subcutaneous fat may either be a marker of visceral fat or may in itself contribute to increased cardiovascular risk in Africans. However, differences in blood pressure did not result in any significant differences between the OW/OB groups for any of the anthropometric or lipid profile variables. On the other hand, the OW/OB NT group could be regarded as a high risk group due to a similar lipid profile and body fat distributions with regard to the OW/OB HT group. With the OW/OB NT group (aged 31.3±1.4

years) being younger ($p \leq 0.07$) than the OW/OB HT group (aged 37.4±2.0 years) may suggest that this group could develop vascular complications in ensuing years and need to be followed up.

CONCLUSION

In the OW/OB HT group, an increase in CO and decrease in vascular function led to the hypertensivity of this group. This seems to be related to a truncal, especially abdominal subcutaneous fat distribution. The decreased vascular function was reaffirmed by the PP exceeding 63mmHg,[35] indicating that this group is at high risk for the development of further cardiovascular complications. Lack of significant differences between the OW/OB groups for the anthropometric and lipid profile variables and the difference in age may indicate that the younger OW/OB NT group is at high risk and should be followed up in ensuing years.

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CHAPTER 3

PLASMA ENDOTHELIN-1 IS NOT INCREASED IN OVERWEIGHT/OBESE HYPERTENSIVE AFRICAN WOMEN: THE *POWIRS* STUDY

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INSTRUCTIONS TO AUTHORS: *Blood Pressure*

- Organise the paper into: 1) Title page, 2) Abstract, 3) Introduction, 4) Materials and Methods, 5) Results, 6) Discussion, 7) Acknowledgements, 8) References.
- Abbreviations and symbols must be standard and SI units should be used throughout.
- The concise Oxford English Dictionary should be used as a reference for spelling and hyphenation.
- The title page should give the title of the paper in no more than 100 characters and should also include a running head not exceeding 45 letter spaces.
- The abstract should include a short summary of the research in no more than 200 words.
- The abstract should also include up to 6 key words.
- State funding and sources of support in the form of grants, equipment or drugs must be acknowledged.
- The Vancouver system of referencing must be applied. References should be numbered consecutively in the order in which they first appear in the paper.
- Abbreviations of journal titles; consult the *List of Journals Indexed in Index Medicus,* published annually as a list in the January issue of *Index Medicus*, also accessible at www.nlm.nih.gov.

SUMMARY

Introduction: Endothelin-1 (ET-1) has been found to be higher in hypertensive African Americans and obese hypertensive Caucasians compared to normotensive controls with an enhanced ET-1-dependent vasoconstrictor tone. ET-1 levels and the associations thereof with cardiovascular function in overweight/obese normotensive and hypertensive African women have not been investigated. It is, therefore, hypothesised that ET-1 levels are elevated in overweight/obese hypertensive African women compared to overweight/obese and lean normotensive controls. Additionally, it is hypothesised that these elevated ET-1 levels are associated with increased total peripheral resistance (TPR) and decreased arterial compliance (C_W). **Materials and Methods:** A case-case control study was performed which included 98 African women. The subjects were divided into lean normotensive (lean NT), overweight/obese normotensive (OW/OB NT) and overweight/obese hypertensive (OW/OB HT). The Finometer apparatus was used to obtain a more elaborate cardiovascular profile and plasma immunoreactive ET-1 levels were determined. **Results:** ET-1 levels were similar for the three groups. Although a decrease in vascular function was observed in the OW/OB HT group, no correlations were obtained between ET-1 and the cardiovascular profile, before and after adjusting for age. **Conclusion:** In African women, ET-1 levels did not differ between lean and overweight/obese and normotensive and hypertensive subjects. The lack of significant associations between ET-1 and decreased vascular function in the overweight/obese hypertensive group suggests that ET-1 is not implicated in obesity-related hypertension in African women.

KEY WORDS: endothelin-1; cardiovascular function, African women

INTRODUCTION

The prevalence of obesity among African women far exceeds those in Caucasian women in South Africa and are approaching that of African American women.[1] However, the prevalence of hypertension in these African women is not the highest compared to the other population groups from South Africa.[2] Survey results have shown that African women have the highest average body mass index (BMI), but the second lowest prevalence of hypertension in comparison with the other population groups i.e., Caucasians, Indians and people of mixed origin.[3]

The high prevalence of hypertension among African Americans prompted Ergul *et al.*[4] to determine whether endothelin-1 (ET-1) possibly plays a role in the etiology of hypertension in African Americans. It was found that ET-1 levels are significantly elevated in hypertensive African American men and women compared to normotensive controls, implicating ET-1 in the development or maintenance of hypertension in this population group. Recently, Campia *et al.*[5] also found that African Americans have an enhanced ET-1-dependent vasoconstrictor tone compared to Caucasians. Additionally, Parrinello *et al.*[6] added obesity in their study on Caucasians and determined that ET-1 levels are significantly elevated in obese hypertensives compared to obese and lean normotensives. Moreover, ET-1 levels were also higher in obese normotensives compared to lean normotensives[6] and Cardillo *et al.*[7] determined that obesity is also associated with an enhanced ET-1-dependent vasoconstrictor activity. Thus, in summary, ET-1 levels are elevated in hypertensive obese Caucasians compared to normotensive obese controls[6] with an enhanced ET-1-dependent vasoconstrictor tone,[7] and elevated in hypertensive African Americans compared to normotensive controls,[4] also with an enhanced ET-1-dependent vasoconstrictor tone.[5]

Apart from the vasoconstrictive effects, ET-1 stimulates vascular smooth muscle cell proliferation and hypertrophy, leading to decreased arterial distensibility and compliance.[8] ET-1 levels and the influence thereof on cardiovascular function have not been determined in African women. It would thus seem relevant to determine whether circulating ET-1 levels are also different in African women with different degrees of adiposity and blood pressure and to determine whether circulating levels are associated with cardiovascular function in these women.

It is, therefore, hypothesised that ET-1 levels are elevated in overweight/obese hypertensive African women compared to overweight/obese and lean normotensive controls. Additionally, it is hypothesised that these elevated ET-1 levels are associated with increased total peripheral resistance (TPR) and decreased arterial compliance (C_W).

MATERIALS AND METHODS

Participants

A case-case control survey was performed which included a sample of 102 urban African women volunteers working at a government institution in the Potchefstroom district of the North West Province, South Africa. A dietician, employed at the institution recruited the subjects according to the initial study design. They had to be apparently healthy African women aged between 20 and 50 years. The dietician attempted to recruit only HIV-negative subjects (according to their status as determined three months prior), but the negative status of all subjects cannot be guaranteed. Subjects were initially recruited based on their BMI as measured at the institution's medical station in lean (BMI:18.5-24.9 kg/m^2), overweight (BMI: 25-29.9 kg/m^2) and obese (BMI: ≥ $30kg/m^2$). Exclusion criteria were pregnancy, lactation, diabetes mellitus and treatment for mental or neurological diseases. The mean age of this group was 31.3 ± 8.6 years. Out of this total sample of 102 subjects, three groups were selected: Firstly, a group of 35 lean normotensive subjects, secondly, a group of 46 normotensive subjects which consisted of a combination of overweight (n=19) and obese (n=27) subjects and thirdly, a group of 17 newly diagnosed hypertensive subjects, also consisting of a combination of overweight (n=6) and obese (n=11) subjects. The subjects were regarded as hypertensive if their blood pressure was ≥ 140 and/or ≥ 90 mmHg.[9] A total of 98 subjects were included in the analysis of this article. The four subjects that were excluded were the only lean hypertensive subjects in the total subject group, rendering the group too small for statistical use.

All research subjects gave informed consent in writing. The Ethics Committee of the North-West University approved the study.

Experimental Procedure

During the course of the study, the subjects reported at a metabolic ward facility (consisting of ten single bedrooms, a living room and kitchen) at 18h00 in the evening. They were all introduced to the experimental set-up including the Finometer apparatus. The purpose of the introduction to the experimental set-up was to minimise anticipation stress and the white coat effect. The subjects received a light meal at about 19h00, which excluded caffeine and alcohol and went to sleep before 23h00. The purpose of the dietary restrictions was to enable a good night's sleep and a stable, resting blood pressure the next morning. Finometer recordings were obtained between 06h00 and 08h00 the next morning, after the overnight rest and before breakfast. Subjects were not permitted to walk around or have anything to eat or drink (except water) until all recordings were completed.

The subject was awake and lying in the Fowler's position in a quiet single bedroom while the Finometer device was connected to the subject. The cuffs was of appropriate size. Blood pressure was recorded continuously for a period of at least seven minutes. After a recording of at least two minutes, the Finometer performed a return-to-flow systolic calibration. This is an individual patient level adjustment which calibrates the upper arm pressure of each specific subject with the finger pressure. Highest precision in blood pressure readings is obtained only after this calibration. From the seven minute continuous blood pressure recording of the Finometer, the average systolic and diastolic blood pressures were determined from the last two minutes of the recording.
The Finometer device computed all cardiovascular variables online and stored the data in result files on a hard disk. The systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), heart rate (HR), stroke volume (SV), cardiac output (CO), TPR, and C_W[10,11] were computed and stored.

The vascular unloading technique of Peñáz together with the Physiocal criteria of Wesseling provided reliable, non-invasive and continuous estimates of blood pressure which are useable especially in comparative studies.[12,13] Since the pressure waveform is available continuously, computations provide further information on the dynamics of the cardiovascular system, similar to intra-arterial measurements.[11,14-18]

Body mass and height measurements were also taken. These anthropometric measurements were performed according to standard methods as described by Norton and Olds.[19] Maximum height was measured to the nearest 0.1 cm, with the head in the Frankfort plane, by means of a stadiometer (Invicta, IP 1465, UK). Body mass was measured to the nearest 0.1 kg by means of a calibrated electronic scale (Precision Health Scale, A&D Company, Japan). During these measurements, the subjects had to stand erect with the feet together and without volitionally contracting the gluteal muscles.

After the Finometer recordings were taken, fasting blood samples were drawn from the vena cephalica or medial cubital vein and plasma was prepared according to standard methods. ET-1 was determined by means of a ^{125}I RIA kit (AEC Amersham (PTY) LTD, Cat No. RPA 545).

Statistical analysis

The computer software package Statistica v/6.0 was used for the analysis of the data. The analysis of covariance (ANCOVA) was used to show significant differences between groups while adjusting for age. The results were seen as statistically significant when the p-value was less or equal to 0.05, which means that each of the three comparisons were tested on a

0.05/3=0.017 level of significance. That is the same as to calculate 98.3% confidence intervals for each of the groups and to determine whether these intervals overlap.[20] Partial correlation coefficients were used to show associations between various variables. A power analysis (80% power) determined that the sample size would be adequate.

RESULTS

Means, standard errors and confidence intervals of cardiovascular and anthropometric variables as well as ET-1 levels are presented in Table 1. Results were adjusted for age due to differences obtained ($p \leq 0.07$) between the three groups.

Table 1: Means ± standard error of cardiovascular and anthropometric variables as well as ET-1 levels adjusted for age at a 98.3% confidence interval.

	Lean NT (n=35)	OW/OB NT (n=46)	OW/OB HT (n=17)
Age (years)	28.3±1.4 [25.7-30.9]	31.3±1.2 [29.1-33.6]	37.4±2.0 [33.7-41.1]
SBP (mmHg)	123±2.3 [118-129]^{a}	124±1.9 [120-129]^{b}	156±1.9 [148-164]^{ab}
DBP (mmHg)	72±1.4 [69-76]^{c}	77±1.2 [74-79]^{d}	91±2.1 [86-96]^{cd}
HR (bts/min)	64.8±1.6 [60.8-68.7]	70.5±1.4 [67.1-73.8]	69.1±2.4 [63.3-74.9]
SV (ml)	80.0±2.4 [74.2-85.9]	87.7±2.1 [82.7-92.7]	89.1±3.5 [80.5-97.7]
CO (l/min)	5.13±0.2 [4.67-5.59]^{ef}	6.10±0.2 [5.71-6.49]^{e}	6.09±0.3 [5.42-6.77]^{f}
TPR (mmHg/ml/s)	1.16±0.04 [1.06-1.25]	0.98±0.04 [0.90-1.06]	1.23±0.06 [1.09-1.37]
C_W(ml/mmHg)	1.78±0.04 [1.68-1.89]^{g}	2.00±0.04 [1.91-2.09]^{gh}	1.65±0.06 [1.49-1.80]^{h}
PP (mmHg)	51.2±1.8 [46.9-55.5]^{i}	47.8±1.5 [44.1-51.4]^{j}	64.7±2.6 [58.4-71.0]^{ij}
ET-1 (fmol/ml)	47.4±7.0 [30.4-64.5]	47.4±6.0 [32.9-62.0]	47.9±10.3 [22.9-73.0]
BMI (kg/m^2)	22.3±0.7 [20.5-24.0]^{kl}	31.3±0.6 [29.8-32.8]^{k}	32.4±1.1 [29.9-35.0]^{l}
Waist circum. (cm)	96.4±1.6 [92.6-100]^{mn}	112±1.3 [109-116]^{m}	114±2.3 [109-120]^{n}

Lean NT (lean normotensive); OW/OB NT (overweight/obese normotensive); OW/OB HT (overweight/obese hypertensive); SBP (systolic blood pressure); DBP (diastolic blood pressure); HR (heart rate); SV (stroke volume); CO (cardiac output); TPR (total peripheral resistance); C_W (Windkessel arterial compliance); PP (pulse pressure); ET-1 (endothelin-1); BMI (body mass index); Waist circum. (waist circumference)

Means with the same superscript: Statistically significant ($p \leq 0.05$)

ET-1 levels were similar for the lean normotensive (lean NT), overweight/obese normotensive (OW/OB NT) and overweight/obese hypertensive (OW/OB HT) groups. Since this result seems surprising, the total group was re-divided into normotensive (NT) (n=81) and hypertensive (HT) (n=17), irrespective of obesity (Table 2). By doing so, again no significant difference could be obtained.

Similarly, no significant difference was obtained when the total group was again re-divided into lean (n=35) and overweight/obese (OW/OB) (n=63), irrespective of blood pressure (Table 2).

Table 2: Means ± standard errors for ET-1 adjusted for age at a 95% confidence interval.

	Normotensive (n=81)	Hypertensive (n=17)
ET-1 (fmol/ml)	47.4±4.5 [38.4-56.4]	47.9±10.2 [27.6-68.3]
	Lean (n=35)	**Overweight/obese (n=63)**
ET-1 (fmol/ml)	47.4±7.0 [33.6-61.3]	47.6±5.2 [37.3-57.8]

ET-1 (endothelin-1)

Apart from the above-mentioned lack of significant differences for ET-1 levels, C_W was significantly lower ($p \leq 0.05$) and TPR higher ($p \leq 0.08$) in the OW/OB HT group compared to the OW/OB NT group. PP was significantly higher in the OW/OB HT group compared to the lean and OW/OB NT groups.

No correlations were obtained between ET-1 and the cardiovascular profile of the total group, lean NT, OW/OB NT and OW/OB HT groups before (Table 3) and after (Table 4) adjusting for age.

Table 3: Partial correlation coefficients between ET-1 and cardiovascular variables.

	Total Group	Lean NT	OW/OB NT	OW/OB HT
	ET-1	ET-1	ET-1	ET-1
SBP	r=0.02; p=0.87	r=-0.23; p=0.17	r=-0.20; p=0.18	r=0.31; p=0.22
DBP	r=-0.08; p=0.42	r=-0.19; p=0.28	r=-0.18; p=0.23	r=0.09; p=0.74
PP	r=0.09; p=0.36	r=-0.07; p=0.67	r=-0.06; p=0.70	r=0.37; p=0.15
TPR	r=-0.03; p=0.75	r=-0.09; p=0.62	r=-0.22; p=0.14	r=0.24; p=0.34
C_W	r=-0.01; p=0.94	r=0.03; p=0.88	r=0.10; p=0.50	r=-0.20; p=0.44

Lean NT (lean normotensive); OW/OB NT (overweight/obese normotensive); OW/OB HT (overweight/obese hypertensive); ET-1 (endothelin-1); SBP (systolic blood pressure); DBP (diastolic blood pressure); PP (pulse pressure); TPR (total peripheral resistance); C_W (Windkessel arterial compliance)

Table 4: Partial correlation coefficients between ET-1 and cardiovascular variables adjusted for age.

	Total Group	Lean NT	OW/OB NT	OW/OB HT
	ET-1	ET-1	ET-1	ET-1
SBP	r=-0.13; p=0.20	r=-0.18; p=0.31	r=-0.24; p=0.12	r=0.28; p=0.33
DBP	r=-0.15; p=0.14	r=-0.19; p=0.28	r=-0.22; p=0.15	r=-0.08; p=0.79
PP	r=-0.03; p=0.75	r=-0.01; p=0.95	r=-0.06; p=0.68	r=0.33; p=0.24
TPR	r=-0.10; p=0.36	r=-0.08; p=0.66	r=-0.22; p=0.15	r=0.39; p=0.17;
C_W	r=0.06; p=0.57	r=-0.02; p=0.90	r=0.19; p=0.21	r=-0.31; p=0.28

Lean NT (lean normotensive); OW/OB NT (overweight/obese normotensive); OW/OB HT (overweight/obese hypertensive); ET-1 (endothelin-1); SBP (systolic blood pressure); DBP (diastolic blood pressure); PP (pulse pressure); TPR (total peripheral resistance); C_W (Windkessel arterial compliance)

DISCUSSION

The results of this study show no differences for ET-1 levels between the lean NT, OW/OB NT and OW/OB HT groups. This was unexpected since Ergul *et al.*[4] demonstrated that ET-1 levels are elevated in hypertensive African American women compared to normotensive controls and Parinello *et al.*[6] found that ET-1 levels are also elevated in obese hypertensive Caucasians compared to obese normotensive controls. Additionally, ET-1 levels were also higher in obese normotensives compared to lean normotensives,[6] indicating that ET-1 levels are elevated possibly as a consequence of human obesity and hypertension. One would thus expect ET-1 levels to be invariably elevated in OW/OB HT hypertensive African women.

Since the results of this study seemed surprising, the total group was re-divided into normotensive and hypertensive, irrespective of obesity and into lean and overweight/obese, irrespective of blood pressure. By doing so, in both cases, still no significant differences could be obtained, suggesting that circulating ET-1 levels are not influenced either by increased adiposity or blood pressure in African women.

A decrease in vascular function in the OW/OB HT group was observed with TPR and PP being higher and C_W significantly lower in the OW/OB HT group compared to the OW/OB NT group. Increased TPR reflects vasoconstriction[21] while decreased C_W and increased PP reflects stiffened arteries,[22] which have both, as mentioned, been found in previous studies to be influenced by circulating ET-1 levels in Caucasian populations.[23,24] Furthermore, the enhanced ET-1-dependent vasoconstrictor tone observed in obese hypertensive Caucasians[7] and hypertensive African Americans[5] would suggest that ET-1 would be associated with the overweight/obese hypertensive African women's increased TPR and/or decreased C_W in this study. Yet no correlations were obtained between ET-1 and these variables. Thus, ET-1, as opposed to hypertensive African American women and obese hypertensive Caucasians, does not seem to play a significant role in obesity-related hypertension in African women.

In conclusion, in African women, ET-1 levels did not differ between lean and overweight/obese, and normotensive and hypertensive subjects. Along with the lack of significant associations between ET-1 and decreased vascular function in the overweight/obese hypertensive group, it is suggested that ET-1 is not implicated in obesity-related hypertension in African women.

Limitations of the study were that the HIV-negative status of the subjects could not be guaranteed as well as the inability to differentiate between the types of hypertension due to a limited number of hypertensive subjects.

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CHAPTER 4

LEPTIN IS INDEPENDENTLY ASSOCIATED WITH SYSTOLIC BLOOD PRESSURE, PULSE PRESSURE AND ARTERIAL COMPLIANCE IN HYPERTENSIVE AFRICAN WOMEN WITH INCREASED ADIPOSITY: THE *POWIRS* STUDY

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Running Title: Leptin and blood pressure in African women

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- The summary should not exceed 250 words and should state concisely what was done, the main findings and how the work was interpreted.
- Three to six keywords should appear at the end of the summary.
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- Abbreviations for titles of medical periodicals should conform to those used in the latest edition of *Index Medicus*.
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- Abbreviations and symbols must be standard and SI units used throughout.
- The Concise Oxford Dictionary is used as a reference for spelling.

SUMMARY

Introduction: High leptin levels are often observed in human obesity and are implicated in obesity-related hypertension. Leptin levels have been found to be higher in hypertensive obese African American women compared to normotensive African American women, but a direct association between leptin and blood pressure could not be obtained. Additionally, increased adiposity has been associated with higher aortic stiffness in obese African American women, but leptin was not included in the study. The effects of leptin on cardiovascular function in African women has not yet been determined. It is hypothesised that leptin is directly associated with blood pressure and decreased arterial compliance (C_W) and that leptin levels are significantly higher in overweight/obese hypertensive African women compared to overweight/obese normotensive African women. **Materials and Methods:** A case-case control study was performed which included 98 African women. The subjects were divided into lean normotensive (lean NT), overweight/obese normotensive (OW/OB NT) and overweight/obese hypertensive (OW/OB HT). The Finometer apparatus was used to obtain a more elaborate cardiovascular profile. Serum leptin and insulin levels as well as the homeostatic assessment model insulin resistance index (HOMA-IR) were determined. Various anthropometric measures were obtained. **Results:** Leptin levels were elevated ($p \leq 0.05$) in the OW/OB NT and HT groups compared to the lean NT group, but were similar in the OW/OB NT and HT groups. After adjusting for obesity, insulin resistance, hyperinsulinemia and age, a direct positive correlation was obtained between leptin and systolic blood pressure (SBP) ($p \leq 0.05$; $r=0.68$) in the OW/OB HT group. Additionally, leptin also correlated negatively with arterial compliance (C_W) ($p \leq 0.05$; $r=-0.76$) and positively with pulse pressure (PP) ($p \leq 0.05$; $r=0.71$) in the OW/OB HT group. **Conclusion:** Even though leptin levels were the same in overweight/obese hypertensive and normotensive African women, leptin was directly and positively associated with SBP and PP and negatively with C_W only in overweight/obese hypertensive African women, independent of obesity, insulin-resistance, hyperinsulinemia and age.

KEY WORDS: leptin, blood pressure, arterial compliance, African women

INTRODUCTION

In African adult populations, little more than a generation ago, rises in weight and blood pressure with age were slight.[1] However, within recent years, in Southern African populations the situation has changed considerably.[2] Obesity is associated with a spectrum of metabolic and cardiovascular disorders[3] and emerges as the single most important risk factor for hypertension in most population surveys,[4] yet the precise contribution of excess weight in causing essential hypertension in different ethnic groups has not been determined.[5]

One of the ways in which excess adiposity could possibly contribute to hypertension is through increased leptin production. Leptin, the *ob* gene product, is predominantly expressed by white adipocytes[6] and correlates well with body fat mass.[7] Not surprisingly, leptin levels are invariably elevated in the obese compared to lean subjects[7] and have been found to be higher in obese hypertensive African American women compared to obese normotensive African American women.[8] Evidence suggests that elevated leptin levels may play an important role in the pathogenesis of obesity-related hypertension[9] and accordingly have been associated with increased blood pressure.[10-15] The mechanisms associating leptin with blood pressure in obesity-related hypertension are multiple. One mechanism has been the involvement of leptin in decreasing vascular function.[16] Leptin has been found to be independently associated with intima-media thickness of the common carotid artery[17] and impaired arterial distensibility, resulting in decreased arterial compliance (C_W).[16] Another possible mechanism is the activation of the sympathetic nervous system by leptin.[18] One of the main consequences of this activation is to cause renal sympathetic nerve activity to increase, leading to sodium retention, volume expansion and increased blood pressure.[19] Additionally, angiotensin II production also increases as a consequence of this activation, increasing plasma levels and resulting in vasoconstriction and blood pressure elevation.[19] However, the association between leptin and blood pressure via sympathetic stimulation was not investigated in this study and the focus will remain on vascular function.

The above-mentioned studies were conducted in non-African population groups. Recently, Wildman *et al.*[20] determined in obese African American women that excess body weight is associated with higher aortic stiffness in subjects between 20 and 30 years of age, but did not include leptin in the study. Since leptin levels are elevated in hypertensive obese African American women compared to normotensive obese controls,[8] one may postulate that leptin could be one of the main contributors to decreased C_W and increased blood pressure in this African American subject group. However, El-Gharbawy *et al.*[8] failed to show a direct association between leptin and blood pressure in obese hypertensive African American women. Because both leptin and hypertension are associated with obesity, insulin resistance and

hyperinsulinemia,[21] El-Gharbawy *et al.*[8] adjusted for these variables in an attempt to obtain this direct association.

Currently, no data is available on the effects of elevated leptin levels on cardiovascular function in hypertensive African women with increased adiposity. Due to the above-mentioned discrepancies in African American women, it is hypothesised that leptin is directly associated with blood pressure and decreased C_W in overweight/obese hypertensive African women, independent of obesity, insulin resistance, hyperinsulinemia and age. Additionally, it is hypothesised that leptin levels are higher in overweight/obese hypertensive African women compared to overweight/obese normotensive African women.

MATERIALS AND METHODS

Participants

A case-case control study was performed which included a sample of 102 apparently healthy African women from a government institution in the North West Province, South Africa. Exclusion criteria were pregnancy, lactation, diabetes mellitus and treatment for mental or neurological diseases requiring the use of any medication. Out of the total sample of 102 subjects, three groups were selected: Firstly, a group of 35 lean normotensive subjects (body mass index (BMI) = 18.5 – 24.9 kg/m^2), secondly, a group of 46 normotensive subjects, which consisted of a combination of overweight (n=19) and obese (n=27) subjects (BMI = 25 – 29.9 and ≥ 30 kg/m^2, respectively) and thirdly, a group of 17 newly diagnosed hypertensive subjects, also consisting of a combination of overweight (n=6) and obese (n=11) subjects. The subjects were regarded as hypertensive if their blood pressure was ≥ 140 and/or ≥ 90 mmHg.[22] A total of 98 subjects were included in the analysis of this article. The four subjects that were excluded were the only lean hypertensive subjects in the total subject group, rendering the group too small for statistical use.

All research subjects gave informed consent in writing. The Ethics Committee of the North-West University approved the study.

Experimental Procedure

During the course of the study, the subjects reported at a metabolic ward facility (consisting of ten single bedrooms, a living room and kitchen) at 18h00 in the evening. They were all introduced to the experimental set-up including the Finometer apparatus. The purpose of the introduction to the experimental set-up was to minimise anticipation stress. The subjects received a light meal at around 19h00, which excluded caffeine and alcohol and went to sleep before 23h00. The purpose of the dietary restrictions was to enable a good night's sleep and a

stable, resting blood pressure the next morning. Finometer recordings were obtained between 06h00 and 08h00 the next morning, after the overnight rest and before breakfast. Subjects were not permitted to walk around or have anything to eat or drink (except water) until all recordings were completed.

The subject was awake and lying in the Fowler's position in a quiet single bedroom while the Finometer device was connected to the subject. Blood pressure was recorded continuously for a period of at least seven minutes. After a recording of at least two minutes, the Finometer performed a return-to-flow systolic calibration. This is an individual patient level adjustment which calibrates the upper arm pressure of each specific subject with the finger pressure. Highest precision in blood pressure readings is obtained only after this calibration. From the seven minute continuous blood pressure recording of the Finometer, the average systolic and diastolic blood pressures were determined from the last two minutes of the recording.

The Finometer device computed all cardiovascular variables online and stored the data in result files on a hard disk. The systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), heart rate (HR), stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR) and C_W were computed and stored.[23,24]

The vascular unloading technique of Peñáz together with the Physiocal criteria of Wesseling provided reliable, non-invasive and continuous estimates of blood pressure which is useable especially in comparative studies.[25,26] Since the pressure waveform is available continuously, computations provide further information on the dynamics of the cardiovascular system, similar to intra-arterial measurements.[24,27-31]

Body mass and height measurements were also taken. These anthropometric measurements were performed according to standard methods as described by Norton and Olds.[32] Maximum height was measured to the nearest 0.1 cm, with the head in the Frankfort plane, by means of a stadiometer (Invicta, IP 1465, UK). Body mass was measured to the nearest 0.1 kg by means of a calibrated electronic scale (Precision Health Scale, A&D Company, Japan). During these measurements the subjects had to stand erect with the feet together and without volitionally contracting the gluteal muscles. Percentage body fat was calculated using the equation of Jackson and Pollock.[33]

After the Finometer recordings were taken, fasting blood samples were drawn from the vena cephalica or medial cubital vein and serum was prepared according to standard methods. Serum leptin levels were measured using a ^{125}I IRMA kit (Diagnostic Systems Laboratories, Inc.,

Cat No. DSL-23100). Analysis of insulin levels was performed by enzyme immunoassay (BioSource EUROPE S.A. Belgium; inter assay CV-7.5%, no cross reactivity with human proinsulin). Serum lipids were determined on a Vitros DT60 II Chemistry System with Vitros DT slides.

Insulin sensitivity was estimated by the homeostatic model assessment (HOMA-IR index), i.e., the product of plasma glucose and insulin, divided by 22.5. Because the group sample obtained was not random, and selected on the basis of BMI, the HOMA-IR index was divided into tertiles. Groups with a HOMA-IR index above the third tertile (3.17) were regarded as insulin resistant.

Statistical analysis

The computer software package Statistica v/6.0 was used for the analysis of the data. The analysis of covariance (ANCOVA) was used to show significant differences between groups while adjusting for age. The results were seen as statistically significant when the p-value was less or equal to 0.05, which means that each of the three comparisons was tested on a 0.05/3=0.017 level of significance. That is the same as to calculate 98.3% confidence intervals for each of the groups and to determine whether these intervals overlap.[34] Partial correlation coefficients were used to show associations between various variables. Linear regression analysis using the forward stepwise method was also used to assess associations between either SBP, DBP, PP, TPR or C_W as dependent variables and leptin, insulin, low-density lipoprotein cholesterol (LDLC), triglycerides and age as independent variables.

RESULTS

Means, standard errors and confidence intervals of cardiovascular and anthropometric variables as well as leptin levels, insulin levels and HOMA-IR indexes are presented in Table 1. Results were adjusted for age due to differences obtained ($p \leq 0.07$) between the overweight/obese hypertensive (OW/OB HT) group and lean normotensive (lean NT) and overweight/obese normotensive (OW/OB NT) groups. Leptin levels were significantly higher in the OW/OB NT and OW/OB HT groups compared to the lean NT group, but leptin values of the OW/OB NT and HT groups were the same. C_W was significantly decreased and PP significantly increased in the OW/OB HT group compared to the OW/OB NT group.

When performing Pearson correlations between variables, leptin correlated positively with adiposity as expected (data not shown). In the lean NT group, leptin correlated with fat % ($p \leq 0.01$; $r=0.54$). In the OW/OB NT group, leptin correlated with BMI ($p \leq 0.01$; $r=0.54$), waist circumference ($p \leq 0.01$; $r=0.58$) and fat % ($p \leq 0.01$; $r=0.57$), and in the OW/OB HT group, leptin correlated with fat % ($p \leq 0.01$; $r=0.53$).

Table 1: Means ± standard errors of cardiovascular variables, leptin, insulin, glucose levels and HOMA-IR indexes. Variables are adjusted for age at a 98.3% confidence interval.

	Lean NT (n=35)	OW/OB NT (n=46)	OW/OB HT (n=17)
Age (years)	28.3±1.4 [25.7-30.9]	31.3±1.2 [29.1-33.6]	37.4±2.0 [33.7-41.1]
SBP (mmHg)	123±2.3 [118-129][a]	124±1.9 [120-129][b]	156±1.9 [148-164][ab]
DBP (mmHg)	72±1.4 [69-76][a]	77±1.2 [74-79][b]	91±2.1 [86-96][ab]
HR (bts/min)	64.8±1.6 [60.8-68.7]	70.5±1.4 [67.1-73.8]	69.1±2.4 [63.3-74.9]
SV (ml)	80.0±2.4 [74.2-85.9]	87.7±2.1 [82.7-92.7]	89.1±3.5 [80.5-97.7]
CO (l/min)	5.13±0.2 [4.67-5.59][ab]	6.10±0.2 [5.71-6.49][a]	6.09±0.3 [5.42-6.77][b]
TPR (mmHg/ml/s)	1.16±0.04 [1.06-1.25]	0.98±0.04 [0.90-1.06]	1.23±0.06 [1.09-1.37]
C_W(ml/mmHg)	1.78±0.04 [1.68-1.89][a]	2.00±0.04 [1.91-2.09][ab]	1.65±0.06 [1.49-1.80][b]
PP (mmHg)	51.2±1.8 [46.9-55.5][a]	47.8±1.5 [44.1-51.4][b]	64.7±2.6 [58.4-71.0][ab]
Leptin (ng/ml)	34.5±3.9 [25.0-43.9][ab]	73.6±3.4 [65.5-81.7][a]	69.8±5.7 [56.0-83.7][b]
Insulin (pmol/l)	76.8±7.0 [61.4-92.2]	105±6.0 [92.7-119]	92.6±10 [69.4-116]
Glucose (mmol/l)	4.88±0.2 [4.39-5.37]	5.22±0.2 [4.80-5.64]	5.85±0.3 [5.14-6.57]
HOMA-IR	2.42±0.3 [1.80-3.04][a]	3.63±0.2 [3.10-4.17][a]	3.10±0.4 [2.16-4.04]
TC (mmol/l)	4.19±0.16 [3.81-4.57]	4.24±0.13 [3.91-4.56]	4.43±0.23 [3.87-4.99]
HDLC (mmol/l)	1.30±0.06 [1.16-1.44]	1.26±0.05 [1.14-1.38]	1.10±0.08 [0.89-1.30]
LDLC (mmol/l)	2.78±0.15 [2.42-3.13]	2.83±0.13 [2.52-3.13]	3.14±0.22 [2.62-3.67]
Trig. (mmol/l)	0.57±0.07 [0.41-0.73][a]	0.73±0.06 [0.59-0.87]	0.97±0.10 [0.73-1.21][a]
BMI (kg/m^2)	22.3±0.7 [20.5-24.0][ab]	31.3±0.6 [29.8-32.8][a]	32.4±1.1 [29.9-35.0][b]
Waist circum. (mm)	96.4±1.6 [92.6-100][ab]	112±1.3 [109-116][a]	114±2.3 [109-120][b]
Fat %	23.4±1.3 [20.4-26.5][ab]	39.4±1.1 [36.8-41.1][a]	41.2±1.8 [36.7-45.7][b]

Lean NT (lean normotensive); OW/OB NT (overweight/obese normotensive); OW/OB HT (overweight/obese hypertensive); SBP (systolic blood pressure); DBP (diastolic blood pressure); HR (heart rate); SV (stroke volume); CO (cardiac output); TPR (total peripheral resistance); C_W (Windkessel arterial compliance); PP (pulse pressure); Leptin (fasting leptin); Insulin (fasting insulin); Glucose (fasting glucose); HOMA-IR (homeostatic model assessment insulin resistance index);TC (total cholesterol); HDLC (high-density lipoprotein cholesterol); LDLC (low-density lipoprotein cholesterol); Trig (triglycerides); BMI (body mass index); Waist circum. (waist circumference); fat % (fat percentage)

Means with the same superscript: Statistically significant ($p \leq 0.05$)

In an attempt to obtain direct associations between leptin and blood pressure, the data were additionally adjusted for obesity, insulin resistance and hyperinsulinemia. Obesity was adjusted for by means of BMI (indicating overall obesity),[35] waist circumference (indicating central or abdominal obesity)[35] and fat % (indicating subcutaneous fat content)[36]. Insulin resistance was adjusted for by means of the HOMA-IR index and hyperinsulinemia by measured serum insulin levels. Results of these partial correlations are shown in Table 2.

After these adjustments, no correlations were obtained between leptin and the cardiovascular variables in the lean NT and OW/OB NT groups. However, in the OW/OB HT group, leptin showed a direct positive correlation with SBP ($p \leq 0.05$; $r=0.68$) and PP ($p \leq 0.05$; $r=0.71$) and a negative correlation with C_W ($p \leq 0.05$; $r=-0.76$).

To support this result, a stepwise regression analysis was performed (Table 3). Using a stepwise linear regression analysis in the OW/OB HT group, with either SBP, DBP, PP, TPR or C_W as dependent variables and leptin, insulin LDLC, triglycerides and age as independent

variables, SBP, PP, TPR and C_W could all be best explained by age, while DBP could be best explained by leptin (Table 3).

Table 2: Partial correlation coefficients between leptin and cardiovascular variables adjusted for obesity, insulin resistance and hyperinsulinemia.

	LEPTIN		
	Lean NT	**OW/OB NT**	**OW/OB HT**
SBP	p=0.26; r=-0.22	p=0.26; r=-0.19	**p≤ 0.05; r=0.68**
DBP	p=0.07; r=-0.35	p=0.53; r=-0.11	p=0.16; r=0.48
PP	p=0.59; r=0.11	p=0.36; r=-0.15	**p≤ 0.05; r=0.71**
HR	p=0.42; r=0.16	p=0.88; r=-0.03	p=0.57; r=0.21
SV	p=0.43; r=-0.16	p=0.86; r=-0.03	p=0.27; r=0.39
CO	p=0.97; r=0.01	p=0.80; r=-0.04	p=0.23; r=0.42
TPR	p=0.37; r=-0.18	p=0.93; r=-0.01	p=0.93; r=0.03
C_W	p=0.67; r=0.09	p=0.40; r=0.14	**p≤ 0.05; r=-0.76**

Lean NT (lean normotensive); OW/OB NT (overweight/obese normotensive); OW/OB HT (overweight/obese hypertensive); Leptin (fasting leptin); SBP (systolic blood pressure); DBP (diastolic blood pressure); PP (pulse pressure); TPR (total peripheral resistance); C_W (Windkessel arterial compliance)

Table 3: Stepwise linear regression analysis partial- and cumulative R^2 values of OW/OB HT group

Independent Variables	Dependent Variables Partial R^2	Cumulative R^2
	SBP	
Age	*0.502*	*0.502*
	DBP	
Leptin	*0.255*	*0.255*
Insulin	0.195	0.450
	PP	
Age	*0.504*	*0.504*
Insulin	0.036	0.540
Leptin	0.038	0.578
	TPR	
Age	0.611	0.611
LDLC	0.030	0.641
Leptin	0.034	0.675
	C_W	
Age	*0.676*	*0.676*

OW/OB HT (overweight/obese hypertensive); SBP (systolic blood pressure); DBP (diastolic blood pressure); TPR (total peripheral resistance); C_W (arterial compliance); LDLC (low density lipoprotein cholesterol)
Independent variable in *italic*: best contributor to R^2

The strong influence of age on the cardiovascular profile as observed in Table 3 supports the adjustment for age when the partial correlations were determined. Due to this strong influence of age, it was accordingly omitted from the multiple regression model (Table 4).

By doing so (Table 4), leptin was the main contributor to SBP and DBP, while insulin was the main contributor to pulse pressure. However, leptin accounted for 17.7% of the variance in PP (R^2=0.504). The main contributors to TPR and C_W were LDLC. Again, leptin was involved and

accounted for 13.9% and 12.8% of the variance in TPR (R^2=0.536) and C_W (R^2=0.464), respectively.

Table 4: Stepwise linear regression analysis partial and cumulative R^2 values of OW/OB HT group with age omitted as independent variable

Independent Variables	Dependent Variables	Cumulative R^2
	Partial R^2	
	SBP	
Leptin	*0.287*	*0.287*
Insulin	0.207	0.494
	DBP	
Leptin	*0.255*	*0.255*
Insulin	0.195	0.450
	PP	
Insulin	*0.260*	*0.260*
Leptin	0.177	0.437
LDLC	0.067	0.504
	TPR	
LDLC	*0.318*	*0.318*
Leptin	0.139	0.457
Insulin	0.079	0.536
Triglycerides	0.083	0.619
	C_W	
LDLC	*0.206*	*0.206*
Leptin	0.128	0.334
Insulin	0.130	0.464

OW/OB HT (overweight/obese hypertensive); SBP (systolic blood pressure); DBP (diastolic blood pressure); TPR (total peripheral resistance); C_W (arterial compliance); LDLC (low density lipoprotein cholesterol)
Independent variable in *italic*: best contributor to R^2

DISCUSSION

Increased leptin levels in the obese have been under close scrutiny over the past decade[37] and have been shown to be an independent predictor of cardiovascular morbidity and mortality.[38-40] However, studies on leptin are limited in African populations[41,42] and the effects of leptin on cardiovascular function in Africans are absent.

Leptin is associated with increased adiposity[7] and accordingly, from the results, leptin levels were higher in the OW/OB NT and HT groups compared to the lean NT group. As expected, positive correlations were obtained between leptin and measures of increased adiposity in all three groups. However, leptin levels were similar between the OW/OB NT and HT groups, which are in contradiction with some studies. Leptin levels have been reported to be higher in patients with hypertension compared to normotensive controls[11,45] and have been positively associated with blood pressure in various non-African population groups.[10-15,45] El-Gharbawy *et al.*[8] failed to obtain a direct positive association between leptin and blood pressure in obese hypertensive African American women with leptin levels that were significantly higher in relation

to an obese normotensive African American female group. Since leptin is independently associated with intima-media thickness,[17] decreased arterial distensibility[16] (decreasing arterial compliance) and blood pressure[45,10-15] in non-African population groups, the findings by El-Gharbawy *et al.*[8] seem surprising since the group under study was hyperleptinemic and hypertensive. Additionally, Wildman *et al.*[20] determined in obese African American women that excess body weight is associated with higher aortic stiffness, but leptin was not included in the study. Thus, one could postulate that leptin could have contributed to the increased aortic stiffness, and hence, decreased C_W.

Table 5. What is known on the topic and what this study adds with regards to leptin and cardiovascular function

What is known about leptin and cardiovascular function	**What this study adds**
a) Leptin correlates with body fat mass and is invariably elevated in obese compared to lean subjects.[7] b) Leptin levels are higher in obese hypertensive African American women compared to obese normotensive African American women.[8] c) Leptin has been found to be positively associated with blood pressure in various population groups,[10-15] but not in Africans. d) Leptin increases blood pressure by: • Decreasing vascular function.[16] Leptin is independently associated with intima media thickness of the common carotid artery[17] and decreased arterial distensibility, decreasing arterial compliance.[16] This is possible since leptin receptors are situated on the endothelium[43] and vascular smooth muscle cells.[44] • Increasing renal sympathetic activity: Results in sodium retention and blood pressure elevation.[19] Increases plasma angiotensin II, increasing vasoconstriction and blood pressure.[19] e) Direct association between leptin and blood pressure could not be obtained in obese hypertensive African American women.[8]	a) This is the first study to investigate leptin's associations with cardiovascular function in an African population group. b) Leptin levels did not differ between the OW/OB NT and OW/OB HT groups, which opposes the finding by El-Gharbawy *et al.*[8] c) Again opposing the findings of El-Gharbawy *et al.*[8] on African American women, leptin is directly and positively associated with SBP and PP and negatively with C_W in overweight/obese hypertensive African women, independent of obesity, insulin-resistance, hyperinsulinemia and age.

El-Gharbawy *et al.*[8] attempted to obtain the above-mentioned direct association between leptin and blood pressure by adjusting for obesity, insulin resistance and hyperinsulinemia, since these variables are associated with both leptin and hypertension.[21] In the present study, a similar route was taken and after these adjustments, no correlations were obtained between leptin and the cardiovascular profile in the lean NT and OW/OB NT groups. However, in the OW/OB HT group, a strong direct positive association was indeed obtained with SBP as well as a strong negative correlation with C_W. This was supported by the positive correlation between leptin and PP, since decreased C_W increases PP and SBP.[46] By performing multiple regression

analyses to confirm the above-mentioned results, leptin showed strong associations with SBP and DBP after omitting age from the multiple regression model. Insulin, along with leptin, both contributed to the increased PP. LDLC, which did not differ significantly between the three groups, was the biggest contributor to the increased TPR and decreased C_W in the OW/OB HT group. However, in both cases, leptin was also a prominent contributor.

The possible influence of leptin on blood pressure via the activation of the sympathetic nervous system cannot be ignored. In this instance, it was not feasible to determine this influence, since sympathetic activity was not measured. However, this influence does not seem probable since the mean heart rates of the three groups were similar, despite the significantly higher leptin levels in the OW/OB groups (Table 1) and the lack of correlations between leptin and HR in the different groups (Table 2).

It seems that leptin does not necessarily have to be elevated in overweight/obese hypertensives compared to overweight/obese normotensives to exhibit its pathological effects. Since leptin receptors are expressed on the endothelium[43] and vascular smooth muscle cells,[44] leptin could possibly affect the function of these cell types and play a pro-atherogenic role in African women, decreasing C_W and increasing PP and SBP.

In conclusion, even though leptin levels were the same in overweight/obese hypertensive and normotensive African women, leptin was directly and positively associated with SBP and PP and negatively with C_W only in overweight/obese hypertensive African women, independent of obesity, insulin-resistance, hyperinsulinemia and age.

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CHAPTER 5

LEPTIN IS FAVOURABLY ASSOCIATED WITH VASCULAR FUNCTION IN OBESE CAUCASIANS, BUT NOT IN OBESE AFRICANS

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Running Title: Leptin and vascular function in Africans and Caucasians

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- Abbreviations and symbols must be standard and SI units used throughout.
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SUMMARY

Introduction: The comparison of the associations between chronically elevated leptin levels and cardiovascular function in obese Africans and Caucasians has not yet been determined. Therefore, the aim of this study was to compare leptin's associations with cardiovascular function in obese African and obese Caucasian women to determine whether leptin's associations differ between these two groups. **Materials and Methods:** This study consisted of two case-case control studies. The first study included a sample of 102 apparently healthy African women and the second, 115 apparently healthy Caucasian women. All lean and obese subjects were selected from each study. The Finometer apparatus was used to obtain a more elaborate cardiovascular profile. Serum leptin levels, insulin levels and the lipid profile were determined. **Results:** Stroke volume (SV) and cardiac output (CO) were significantly ($p \leq 0.01$) elevated in both obese African and Caucasian groups compared to their lean controls. Total peripheral resistance (TPR) was significantly decreased and arterial compliance (C_W) significantly increased in both obese African and Caucasian groups. In the obese Caucasian group, diastolic blood pressure (DBP) was significantly ($p \leq 0.01$) lower, SV and C_W significantly higher ($p \leq 0.01$) and TPR significantly lower compared to the age, BMI, and leptin-matched obese African group. After adjusting for age and body mass index (BMI), leptin correlated negatively with DBP ($p \leq 0.05$; $r=-0.33$) and TPR ($p \leq 0.05$; $r=-0.36$) in the obese Caucasian group, but not in the obese African group. **Conclusions:** Even though leptin levels were similar in obese African and Caucasian women, leptin is favourably associated with vascular function in obese Caucasians, but not in obese Africans.

KEY WORDS: Leptin, obesity, cardiovascular function, Africans, Caucasians

INTRODUCTION

The hemodynamic profile of obese normotensive subjects is characterized by a high intravascular volume, high stroke volume (SV) and cardiac output (CO) and normal or reduced total peripheral resistance (TPR).[1,2] The eventual transition from a normotensive to a hypertensive state normally results from structural changes in the peripheral vasculature,[3] increasing TPR[4] and decreasing arterial compliance (C_W).[5] Since the adipocyte derived hormone, leptin, is invariably elevated in the obese,[6] studies have suggested the involvement of leptin in obesity-related hypertension.[7] Rightly so, leptin has pressor actions and has been associated with blood pressure[8-13] as well as decreased vascular function,[14,15] lowering C_W and increasing TPR. Furthermore, leptin also activates the sympathetic nervous system.[16] This activation increases renal sympathetic nerve activity, leading to sodium retention, volume expansion and increased blood pressure.[17] Additionally, angiotensin II production also increases as a consequence of this activation, increasing plasma angiotensin II levels and resulting in vasoconstriction, increasing TPR and decreasing C_W.[17] Apart from leptin's pressor actions, leptin also has depressor actions. Leptin has been shown, after acute administration, to cause vasodilation through nitric oxide dependent and independent mechanisms, decreasing TPR, increasing C_W, and decreasing blood pressure.[18-20]

The above-mentioned studies determined that leptin has pressor and depressor effects that would result in decreased or increased vascular function and elevated or reduced blood pressure as serum leptin levels increase with increased adiposity. Associations of chronically elevated leptin levels with cardiovascular function in obese African women are limited[21] and comparisons of leptin's associations with cardiovascular function between Africans and Caucasians absent. Recently, it was determined that leptin is independently associated with increased systolic blood pressure (SBP) and decreased C_W in a group of overweight/obese hypertensive African women.[21] The aim of this study was to compare leptin's associations with cardiovascular function in obese African and obese Caucasian women to determine whether leptin's associations differ between these two groups.

MATERIALS AND METHODS

Participants

This study consisted of two case-case control studies. The first study included a sample of 102 apparently healthy African women and the second, 115 apparently healthy Caucasian women from the North West Province, South Africa. Exclusion criteria were pregnancy, lactation, diabetes mellitus and treatment for mental or neurological diseases requiring the use of any medication. Out of the total sample of 102 African subjects, all lean subjects (n=39) (body mass index (BMI) = 18.5 – 24.9 kg/m^2),[22] and all obese subjects (n=38) (BMI ≥ 30 kg/m^2)[22] were

selected. Similarly, out of the total sample of 115 Caucasian subjects, all lean (n=42) and obese subjects (n=41) were selected.

All research subjects gave informed consent in writing. The Ethics Committee of the North-West University approved the study.

Experimental Procedure

During the course of the study, the subjects reported at a metabolic ward facility (consisting of ten single bedrooms, a living room and kitchen) at 18h00 in the evening. They were all introduced to the experimental set-up including the Finometer apparatus. The purpose of the introduction to the experimental set-up was to minimise anticipation stress. The subjects received a light meal at about 19h00, which excluded caffeine and alcohol and went to sleep before 23h00. The purpose of the dietary restrictions was to enable a good night's sleep and a stable, resting blood pressure the next morning. Finometer recordings were obtained between 06h00 and 08h00 the next morning, after the overnight rest and before breakfast. Subjects were not permitted to walk around or have anything to eat or drink (except water) until all recordings were completed.

The subject was awake and lying in the Fowler's position in a quiet single bedroom while the Finometer device was connected to the subject. Blood pressure was recorded continuously for a period of at least seven minutes. After a recording of at least two minutes, the Finometer performed a return-to-flow systolic calibration. This is an individual patient level adjustment which calibrates the upper arm pressure of each specific subject with the finger pressure. Highest precision in blood pressure readings is obtained only after this calibration. From the seven minute continuous blood pressure recording of the Finometer, the average systolic and diastolic blood pressures were determined from the last two minutes of the recording.

The Finometer device computed all cardiovascular variables online and stored the data in result files on a hard disk. The SBP, diastolic blood pressure (DBP), pulse pressure (PP), heart rate (HR), SV, CO, TPR, and C_W were computed and stored.[23,24]

The vascular unloading technique of Peñáz together with the Physiocal criteria of Wesseling provided reliable, non-invasive and continuous estimates of blood pressure which are useable especially in comparative studies, as with this one.[25,26] Since the pressure waveform is available continuously, computations provide further information on the dynamics of the cardiovascular system, similar to intra-arterial measurements.[24,27-31]

Body mass and height measurements were also taken. These anthropometric measurements were performed according to standard methods as described by Norton and Olds.[32] Maximum height was measured to the nearest 0.1 cm, with the head in the Frankfort plane, by means of a stadiometer (Invicta, IP 1465, UK). Body mass was measured to the nearest 0.1 kg by means of a calibrated electronic scale (Precision Health Scale, A&D Company, Japan). During these measurements the subjects had to stand erect with the feet together and without volitionally contracting the gluteal muscles.

After the Finometer recordings were taken, fasting blood samples were drawn from the vena cephalica or medial cubital vein and serum was prepared according to standard methods. Serum leptin levels were measured using a ^{125}I IRMA kit (Diagnostic Systems Laboratories, Inc., Cat No. DSL-23100). Analysis of insulin levels was performed by enzyme immunoassay (BioSource EUROPE S.A. Belgium; no cross reactivity with human proinsulin). Serum lipids were determined on a Vitros DT60 II Chemistry System with Vitros DT slides.

Statistical analysis

The computer software package Statistica v/7.0 was used for the analysis of the data. The student t-test and the analysis of covariance (ANCOVA) were used to show significant differences between groups. Partial correlations coefficients were used to show associations between various variables. Linear regression analysis using the forward stepwise method was also used to assess associations between either SBP, DBP, SV, CO, TPR or C_W as dependent variables and leptin, BMI, insulin, low-density lipoprotein cholesterol (LDLC), triglycerides and age as independent variables.

RESULTS

Means, standard errors and confidence intervals of cardiovascular variables, BMI, age and leptin are presented in Table 1.

SV was significantly ($p \leq 0.01$) elevated in both the African and Caucasian obese groups compared to the lean African and Caucasian controls, respectively. Similarly, TPR was significantly ($p \leq 0.01$) decreased and C_W significantly ($p \leq 0.01$) increased in the obese African and Caucasian groups. Leptin was significantly ($p \leq 0.01$) elevated in both African and Caucasian obese groups compared to their lean controls. The above-mentioned comparisons were adjusted for age due to differences obtained between the lean and obese African ($p \leq 0.01$) and lean and obese Caucasian groups ($p \leq 0.01$).

By comparing Africans and Caucasians (Table 1), leptin levels, BMI and age were found to be similar for the obese African and Caucasian groups. However, DBP was significantly ($p \leq 0.01$) lower in the obese Caucasian group. SV was significantly ($p \leq 0.01$) higher, TPR significantly ($p \leq 0.01$) lower and C_W significantly ($p \leq 0.01$) higher in the obese Caucasian group compared to the obese African group. Similar trends were observed by comparing the lean African and Caucasian groups, except for leptin which was significantly ($p \leq 0.05$) higher in the lean African group.

Table 1: Means ± standard errors of variables from the lean African and Caucasian group at a 95% confidence interval.

LEAN

	AFRICANS (n=39)	CAUCASIANS (n=42)	p-values between lean Africans and Caucasians
Age (years)	28.5±1.2 [26.1-30.9]	28.3±1.3 [25.6-30.9]	p=0.90
BMI (kg/m^2)	21.9±0.3 [21.4-22.4]	21.8±0.3 [21.1-22.5]	p=0.80
SBP (mmHg)	123±1.7 [120-127]	121±1.6 [118-124]	p=0.37
DBP (mmHg)	73±1.3 [70-76]	68±1.3 [65-71]	**p≤ 0.01**
PP (mmHg)	50.5±1.4 [47.8-53.2]	53.3±1.3 [50.6-56.0]	p=0.15
HR (bts/min)	65.2±1.4 [62.3-68.1]	71.5±1.6 [68.4-74.7]	**p≤ 0.01**
SV (ml)	79.9±2.3 [75.3-84.5]	86.7±3.1 [80.5-92.8]	p=0.08
CO (l/min)	5.15±0.2 [4.81-5.50]	6.07±0.2 [5.70-6.45]	**p≤ 0.01**
TPR (mmHg/ml/s)	1.16±0.04 [1.07-1.25]	0.91±0.03 [0.86-0.97]	**p≤ 0.01**
C_W(ml/mmHg)	1.80±0.04 [1.72-1.87]	2.07±0.05 [1.97-2.17]	**p≤ 0.01**
Leptin (ng/ml)	32.1±2.2 [27.6-36.6]	24.3±2.9 [18.5-30.1]	**p≤ 0.05**

OBESE

	AFRICANS (n=38)	p-values between lean and obese Africans*	CAUCASIANS (n=41)	p-values between lean and obese Caucasians*	p-values between obese Africans and Caucasians
Age (years)	34.4±1.5 [31.5-37.4]	N/A	34.1±1.4 [31.4-36.8]	N/A	p=0.87
BMI (kg/m^2)	34.8±0.7 [33.4-36.2]	**p≤ 0.01**	36.1±0.8 [34.4-37.8]	**p≤ 0.01**	p=0.24
SBP (mmHg)	136±4.2 [128-145]	p=0.10	128±1.7 [125-132]	**p≤ 0.01**	p=0.08
DBP (mmHg)	83±1.7 [79-86]	**p≤ 0.01**	76±1.2 [73-78]	**p≤ 0.01**	**p≤ 0.01**
PP (mmHg)	53.5±2.8 [47.7-59.2]	p=0.74	52.8±1.6 [49.7-56.0]	p=0.64	p=0.84
HR (bts/min)	70.3±1.7 [66.9-73.7]	p=0.07	75.2±1.1 [72.9-77.4]	**p≤ 0.05**	**p≤ 0.05**
SV (ml)	90.2±2.3 [84.5-94.9]	**p≤ 0.01**	115±4.3 [106-124]	**p≤ 0.01**	**p≤ 0.01**
CO (l/min)	6.25±0.2 [5.89-6.61]	**p≤ 0.01**	8.54±0.3 [7.91-9.16]	**p≤ 0.01**	**p≤ 0.01**
TPR (mmHg/ml/s)	1.04±0.04 [0.96-1.12]	**p≤ 0.01**	0.71±0.03 [0.66-0.77]	**p≤ 0.01**	**p≤ 0.01**
C_W(ml/mmHg)	1.92±0.06 [1.80-2.04]	**p≤ 0.01**	2.55±0.06 [2.42-2.67]	**p≤ 0.01**	**p≤ 0.01**
Leptin (ng/ml)	83.4±4.1 [75.1-91.6]	**p≤ 0.01**	81.3±4.1 [72.9-89.6]	**p≤ 0.01**	p= 0.72

SBP (systolic blood pressure); DBP (diastolic blood pressure); PP (pulse pressure); HR (heart rate); SV (stroke volume); CO (cardiac output); TPR (total peripheral resistance); C_W (Windkessel arterial compliance); BMI (body mass index); Leptin (fasting leptin).

*: Age-adjusted

Due to the influence of age within each group, partial correlations were performed while adjusting for age. Leptin correlated positively with BMI ($p \leq 0.01$; $r=0.69$), SV ($p \leq 0.01$; $r=0.42$), CO ($p \leq 0.01$; $r=0.49$) and C_W ($p \leq 0.01$; $r=0.45$), and negatively with TPR ($p \leq 0.01$; $r=-0.52$) in the obese Caucasian group. Similarly, in the lean Caucasian group, leptin also correlated positively

with BMI (p≤ 0.01; r=0.80), CO (p≤ 0.05; r=0.35) and C_W (p≤ 0.01; r=0.49). Apart from the positive correlation between leptin and BMI (p≤ 0.05; r=0.34) in the obese African group, no correlations could be obtained between leptin and the cardiovascular profile of both lean and obese African groups after adjusting for age.

Table 2: Partial correlation coefficients between leptin and cardiovascular variables adjusted for age and BMI.

	LEPTIN			
	Lean Africans	**Lean Caucasians**	**Obese Africans**	**Obese Caucasians**
SBP	p=0.61; r=-0.09	p=0.66; r=-0.08	p=0.69; r=-0.07	p=0.71; r=-0.06
DBP	p=0.09; r=-0.28	p=0.78; r=-0.05	p=0.75; r=-0.06	p≤ 0.05; r=-0.33
PP	p=0.29; r=0.18	p=0.83; r=-0.04	p=0.71; r=-0.07	p=0.30; r=0.17
HR	p=0.29; r=0.18	p=0.95; r=-0.01	p=0.08; r=0.30	p=0.58; r=0.10
SV	p=0.80; r=0.04	p=0.13; r=0.48	p=0.37; r=-0.16	p=0.14; r=0.25
CO	p=0.34; r=0.16	p=0.53; r=0.11	p=0.39; r=0.15	p=0.10; r=0.28
TPR	p=0.13; r=-0.25	p=0.51; r=-0.12	p=0.53; r=-0.11	p≤ 0.05; r=-0.36
C_W	p=0.26; r=0.19	p=0.25; r=0.21	p=0.56; r=0.10	p=0.64; r=0.08

Leptin (fasting leptin); SBP (systolic blood pressure); DBP (diastolic blood pressure); PP (pulse pressure); HR (heart rate); SV (stroke volume); TPR (total peripheral resistance); C_W (Windkessel arterial compliance)

Due to BMI's positive association with leptin in both obese Caucasian and obese African groups, as well as BMI's similar associations as leptin with SV (Caucasians: p≤ 0.05, r=0.38; Africans: p≤ 0.01, r=0.65), CO (Caucasians: p≤ 0.01, r=0.49; Africans: p≤ 0.01, r=0.52), C_W (Caucasians: p≤ 0.01, r=0.59; Africans: p≤ 0.01, r=0.51) and TPR (Caucasians: p≤ 0.01, r=-0.43; Africans: p≤ 0.05, r=-0.32), the data were additionally adjusted for BMI. By doing so, leptin correlated negatively with DBP and TPR only in the obese Caucasian group (Table 2).

To support these results, a stepwise regression analysis was performed (Table 3). By performing this analysis in the obese Caucasian group, with either SBP, DBP, SV, CO, TPR or C_W as dependent variables and leptin, BMI, age, LDLC, triglycerides and insulin as independent variables, leptin was the strongest contributor to the decreased DBP and TPR in the obese Caucasian group, while BMI was the strongest contributor to the increased SV, CO and C_W.

Table 3: Stepwise linear regression analysis partial R^2 and Beta values of Caucasian and African obese groups.

OBESE CAUCASIANS			OBESE AFRICANS		
Independent Variables	**Dependent Variables Partial R^2**	**Beta**	**Independent Variables**	**Dependent Variables Partial R^2**	**Beta**
	SBP			**SBP**	
-	-	-	*Age*	*0.21*	*0.463*
	DBP			**DBP**	
Age	0.036	-0.350	-	-	-
BMI	0.038	0.480	-	-	-
Leptin	*0.100*	*-0.380*	-	-	-
LDLC	0.028	0.174	-	-	-
	SV			**SV**	
BMI	*0.175*	*0.139*	*BMI*	*0.400*	*0.674*
Age	0.044	0.276	Age	0.049	-0.261
Leptin	0.047	0.298	Insulin	0.031	-0.169
Insulin	0.026	0.165	LDLC	0.019	0.148
	CO			**CO**	
BMI	*0.264*	*0.256*	*BMI*	*0.265*	*0.489*
Leptin	0.037	0.335	Insulin	0.025	0.211
Age	0.037	0.185	Triglycerides	0.025	-0.169
Triglycerides	0.023	0.155	-	-	
	TPR			**TPR**	
Leptin	*0.254*	*-0.531*	*Age*	*0.162*	*0.411*
Age	0.062	-0.224	BMI	0.088	-0.229
Trig	0.027	-0.167	Insulin	0.038	-0.207
	C_W			**C_W**	
BMI	*0.216*	*0.484*	*Age*	*0.349*	*-0.606*
Age	0.213	-0.424	BMI	0.167	0.409
LDLC	0.052	-0.269	-	-	-
Insulin	0.036	0.197	-	-	-

SBP (systolic blood pressure); DBP (diastolic blood pressure); SV (stroke volume); CO (cardiac output); TPR (total peripheral resistance); C_W (arterial compliance); LDLC (low density lipoprotein cholesterol); Leptin (fasting leptin); Insulin (fasting insulin)
Independent variable in *italic*: best contributor to R^2

DISCUSSION

The typical volume-loading effect associated with obesity[1,2] was observed in both obese African and Caucasian groups with the SV and CO increasing significantly in these groups compared to their lean controls (Table 1). Also, the accommodating effect of the vasculature due to the increased intravascular volume[1,2] was also presented by both obese African and Caucasian groups, with the TPR decreasing and C_W increasing significantly compared to their lean controls. As expected, leptin was significantly elevated in both obese African and Caucasian groups compared to their lean controls, since leptin, as a product of adipose tissue, is invariably elevated in the obese.[6]

Even though serum leptin levels, BMI and age were similar for both African and Caucasian obese groups, DBP was significantly lower in the obese Caucasian group. Supporting the lower DBP was the significantly lower TPR and significantly higher C_W,[33] indicating normal or enhanced vascular functioning associated with obesity in this group of obese Caucasians. A decreased TPR would increase SV,[34] which was indicated by the significantly decreased TPR and increased SV in the obese Caucasian group compared to the obese African group. Thus, the accommodating effect due to volume loading associated with obesity,[1,2] as observed in both obese African and Caucasian groups, seems to be more prominent in the obese Caucasian group, explaining the lower DBP compared to the obese African group. On the other hand, the obese African group in turn could be regarded as having an impaired accommodating effect to the observed volume loading, explaining the higher DBP compared to the obese Caucasian group.

Table 4. What is known on the topic and what this study adds with regards to leptin and cardiovascular function

What is known about leptin and cardiovascular function	**What this study adds**
a) Leptin correlates with body fat mass and is invariably elevated in obese compared to lean subjects.[6] b) Leptin has been found to be positively associated with blood pressure in various population groups.[8-13] c) Leptin is independently associated with systolic blood pressure, pulse pressure and arterial compliance in obese hypertensive African women.[21] d) Leptin has pressor effects by: • Decreasing vascular function.[14] Leptin is independently associated with intima media thickness of the common carotid artery[15] and decreased arterial distensibility, decreasing arterial compliance.[14] This is possible since leptin receptors are situated on the endothelium[35] and vascular smooth muscle cells.[36] • Increasing renal sympathetic activity: Results in sodium retention and blood pressure elevation.[19] Increases plasma angiotensin II, increasing vasoconstriction and blood pressure.[19] e) Leptin has depressor effects by causing vasodilation through nitric oxide dependent and independent mechanisms.[18-20]	a) This is the first study to compare the associations of chronically elevated leptin levels with cardiovascular function between obese African and Caucasian women. b) Chronically elevated leptin levels are favourably associated with vascular function in obese Caucasian women, but not in obese African women.

Since leptin's positive[18-20] and negative[14,15] influences on vascular function has been reported, partial correlations were performed while adjusting for age to determine if leptin is associated with the obese African group's possible decreased vascular function, or the obese Caucasian group's possible normal or enhanced vascular function. By doing so, leptin failed to show any

associations with the vascular profile in the obese African group. However, leptin correlated positively with SV and CO in the obese Caucasian group. Additionally, leptin correlated negatively with TPR and positively with C_W, implicating leptin as a possible role player in the accommodating effect observed in the obese Caucasian group, but not in the obese African group. One may speculate that this accommodating effect could be the result of leptin's angiogenic[37,38] and/or vasodilatory[18-20] effect, which would lead to decreased TPR and increase C_W.

These associations observed in the obese Caucasian group could merely be due to leptin's association with BMI, which showed similar associations with SV, CO, TPR and C_W. To test this possibility, the data was additionally adjusted for BMI (Table 2). Indeed, after this adjustment, the associations between leptin and SV, CO and C_W disappeared, but a negative association with DBP became apparent and the negative association with TPR remained. These results were confirmed by means of multiple regression analyses (Table 3). The lack of associations between leptin and vascular function in the obese African group, implicates leptin to have a positive influence on vascular function in obese Caucasians, but not in obese Africans. This lack of a positive influence of leptin on vascular function in African women is supported by results from a recent publication,[21] which showed leptin to be independently associated with SBP and decreased C_W in obese hypertensive African women. Since the SBP and DBP of both obese African and Caucasian groups were in the normotensive range,[39] the presence of prominent positive associations between leptin and vascular function in the obese Caucasians and the lack thereof in the obese Africans, could indicate that leptin predominantly exerts pathological influences on vascular function in obese African women, as determined previously in obese hypertensive African women.[21]

In conclusion, even though leptin levels were similar in obese African and Caucasian women, leptin was favourably associated with vascular function in obese Caucasians, but not in obese Africans.

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CHAPTER 6

GENERAL FINDINGS AND CONCLUSIONS

1. INTRODUCTION

In this chapter, a summary of the main findings from the four articles reported in this thesis will be given. The results from each article will be discussed, interpreted, elucidated and compared to the relevant literature in the separate chapters. Conclusions will be drawn and recommendations will be made to researchers investigating obesity-related hypertension in African women from South Africa.

2. SUMMARY OF MAIN FINDINGS

The salient findings of the four aspects reported in this thesis were:

2.1 Cardiovascular function in African women with different BMI's and blood pressures (Chapter 2)

The aims of the first aspect of the study were to compare the cardiovascular profiles of African women with different body mass indexes (BMI's) and blood pressures and to describe possible adverse influences to normal cardiovascular functioning in this group. It was, therefore, hypothesised that the cardiovascular profile of overweight/obese hypertensive African women is characterised by an elevated cardiac output (CO), increased total peripheral resistance (TPR) and decreased arterial compliance (C_W) and that the detrimental vascular profile is associated with a truncal fat distribution.

Results indicated that the overweight/obese normotensive (OW/OB NT) and overweight/obese hypertensive (OW/OB HT) groups showed a volume loading effect compared to the lean normotensive (lean NT) group. A decrease in vascular function was observed in the OW/OB HT group with the TPR being increased and C_W decreased compared to the OW/OB NT group. The first hypothesis is thus accepted.

In the total group, the abdominal skinfold showed positive associations with blood pressure and after adjusting for body mass index (BMI) and waist circumference, correlated negatively (unfavourably) with vascular function, i.e. correlated positively with TPR and negatively with C_W. Additionally, in the OW/OB HT group, the abdominal skinfold also showed a positive association with TPR. The second hypothesis is thus accepted.

2.2 Plasma endothelin-1 in overweight/obese hypertensive African women (Chapter 3)

The aims of the second aspect of the study were to investigate endothelin-1 (ET-1) levels in African women with different levels of adiposity and blood pressures and to establish whether associations exist between ET-1 and vascular function in OW/OB HT African women. It was hypothesised that ET-1 levels are increased in OW/OB HT African women compared to lean NT and OW/OB NT African women and that ET-1 is positively associated with TPR and negatively associated with C_W in OW/OB HT African women.

Plasma ET-1 levels were unexpectedly similar for the lean NT, OW/OB NT and OW/OB HT groups. Since this seemed surprising, the total group was re-divided into normotensive and hypertensive, irrespective of obesity. After doing so, still no difference could be obtained. Similarly, by again re-dividing the group into lean and overweight/obese, irrespective of blood pressure, no differences could be obtained between the groups. Additionally, no correlations could be obtained between ET-1 and the cardiovascular profile of the total, lean NT, OW/OB NT and OW/OB HT groups, resulting in the rejection of the first and second hypotheses.

2.3 Leptin is independently associated with systolic blood pressure, pulse pressure and arterial compliance (Chapter 4)

The aims of the third aspect of the study were to investigate leptin levels in African women with different levels of adiposity and blood pressures and to determine if leptin is directly associated with blood pressure and decreased C_W. It was hypothesised that leptin levels are higher in OW/OB HT African women compared to OW/OB NT African women and that leptin is independently associated with blood pressure and decreased C_W in OW/OB HT African women, independent of obesity, insulin resistance, hyperinsulinemia and age.

Leptin levels were, as expected, elevated in both OW/OB NT and HT groups compared to the lean NT group, but did not differ between the OW/OB NT and OW/OB HT groups. The first hypothesis is thus rejected. Leptin was directly and positively associated with systolic blood pressure (SBP) and pulse pressure (PP) and negatively with C_W in the OW/OB HT group after adjusting for obesity, insulin-resistance, hyperinsulinemia and age, resulting in the acceptance of the second hypothesis.

2.4 Leptin is favourably associated with vascular function in obese Caucasians, but not in Africans (Chapter 5)

The aim of the fourth aspect of the study was to compare leptin's associations with cardiovascular function in obese African and obese Caucasian women to determine whether leptin's associations differ between these two groups. It was hypothesised that high serum leptin levels are adversely associated with vascular function in obese African and obese Caucasian women.

By comparing the obese groups with their lean controls, the expected volume loading effect associated with obesity was observed in both obese Caucasian and African groups, as well as the corresponding accommodating effect, that is, decreased TPR and increased C_W. Even though leptin, BMI and age were similar for both African and Caucasian obese groups, the accommodating effect seemed to be more prominent in the Caucasian group, explaining the lower diastolic blood pressure (DBP). Leptin was negatively associated with DBP and TPR in the obese Caucasian group, but not in the obese African group. Thus, leptin was favourably associated with vascular function in obese Caucasians, but not in obese Africans. However, no adverse associations were obtained between leptin and decreased vascular function in either the obese African or Caucasian groups, resulting in the rejection of the hypothesis.

3. COMPARISON OF FINDINGS WITH THE LITERATURE

When the results from this study (Chapters, 2, 3, 4, 5) are compared with results found in the literature regarding other population groups, it is evident that certain findings confirmed and others contradicted those found in the literature, but also added to the available literature. Confirming findings were the cardiovascular profile of the OW/OB HT group that was similar to other obese hypertensive groups found in the literature (Zhang & Reisin, 2000; Taler *et al.*, 2004; Wildman *et al.*, 2003) as well as the abdominal area (subcutaneous) that was associated with blood pressure and decreased vascular function (Misra & Vikram, 2003; Plavnic *et al.*, 2001). Also, the leptin levels that were elevated in the OW/OB NT and HT groups compared to the lean NT group were in accordance with the literature (Considine *et al.*, 1996).

Contradictory findings of this study were that ET-1 levels were similar for the different group subdivisions, while Ergul *et al.*, (1996) found ET-1 levels to be elevated in

hypertensive African American men and women compared to normotensive controls, as well as Parrinello *et al.* (1996) who found ET-1 levels to be elevated in obese hypertensive Caucasians compared to obese and lean normotensives. Thus it seems from these studies that ET-1 levels are elevated when the obesity and/or hypertension component is present, which makes it controversial that no differences could be obtained in African women in which both hypertension and obesity were present. Similarly, the fact that no differences in leptin levels between the OW/OB NT and OW/OB HT groups could be obtained was also a contradictory finding, since El-Gharbawy *et al.* (2002) found leptin levels to be higher in obese hypertensive African American women compared to obese normotensive African American women.

A result that added to the available literature was the independent association that was obtained between leptin and blood pressure which EL-Gharbawy *et al.* (2002) failed to obtain in African American women. Additionally, leptin showed independent associations with C_W and PP which has not been published before in Africans. Leptin was also found to be favourably associated with vascular function in obese Caucasians, but not in obese Africans. Comparisons of the associations of chronically elevated leptin levels with cardiovascular function in obese Caucasian and African women have not been published to date.

4. CHANCE AND CONFOUNDING

Before the main findings of this study are discussed, it is important to reflect critically on some important factors that may have affected the results. There are some methodological issues that could have caused weaknesses in this study and, therefore, might have influenced the different outcomes.

At first, the number of subjects included in this study could be questioned, especially the number of subjects in the OW/OB HT group (n=17). The reason for this was that subjects were selected on the basis of BMI into relatively equal lean, overweight and obese groups, without considering hypertensive status. Although a statistical power analysis determined that 15 subjects would be sufficient, this was determined by using blood pressure. Leptin or endothelin-1 levels could not be used to determine power due to the variability of the different test kits used in different studies. In Chapters 2, 3 and 4, 98 of the 102 subjects were used due to 4 lean hypertensive subjects that were present. This group was too small for statistical use. It would have been ideal to have this group for comparison also.

Concerning the results, the possibility of chance should be taken into account. By using partial correlations and forward stepwise regression analysis, statistics indicated that one out of twenty significant correlations may be because of chance.

Confounders

Confounders such as smoking, alcohol intake, level of physical activity, socio-economic status and HIV-status could have influenced the results by causing over or under-estimation of the associations between cardiovascular function and the various variables investigated in this study. The subjects included were tested for HIV three months prior to this study. Those who were positive were excluded during the recruitment phase. However, the possibility existed that some of the subjects could have been infected during the three month period and ethical issues prevented further testing. Age, as a possible confounder, was addressed by statistically adjusting for it.

In the interpretation of the results in this thesis, it was attempted to interpret statistical results from a physiological standpoint at all times, while keeping in mind that a statistical significance does not necessarily mean physiological significance, and *vice versa*.

5. DISCUSSION OF MAIN FINDINGS

Obesity in African women is traditionally seen with less disfavour compared to other population groups (Walker *et al.*, 2001). Thus, it is considered acceptable, or even desirable and "healthy" for African women to be obese, leaving these women with little or no incentive to lose weight. The rate of urbanisation has increased drastically over the past decade, resulting in the adoption of a more Westernised lifestyle. This in turn was and is resulting in dramatic increments in the prevalence of obesity and the development of cardiovascular disease (Van Rooyen *et al.*, 2000).

Although it is difficult to generalise to the whole African female population of South Africa, findings of this study would serve as a point of departure for larger scale studies to follow, in order to combat obesity-related hypertension and the development of cardiovascular disease.

The main focus of this study was directed towards adverse influences on vascular function in the obese by investigating associations of various anthropometric and endocrinological variables with decreased vascular function.

The decreased vascular function was observed in the OW/OB HT group. TPR was increased and a clear decrease in C_W could be observed in comparison to the OW/OB NT group, which was confirmed by a high PP, exceeded 63 mmHg, which was determined to be associated with subsequent cardiovascular complications (Fang *et al.*, 1995). This decrease in vascular function was associated with a truncal, especially an abdominal fat distribution. Since this fat distribution was subcutaneous, it may suggest that abdominal subcutaneous fat may either be a marker of visceral fat, or may in itself contribute to increased cardiovascular risk in Africans. This however, needs to be confirmed.

The endocrinological factors investigated in this study were ET-1 and leptin due to ET-1's prominent role in hypertension in African Americans (Ergul *et al.*, 1996; Ergul *et al.*, 1999; Campia *et al.*, 2004), obesity-related hypertension in Caucasians (Parrinello *et al.* 1996) and also leptin's association with obesity-related hypertension in African Americans (El-Gharbawy *et al.*, 2002) and decreased vascular function in Caucasians (Singhal *et al.*, 2002; Ciccone *et al.*, 2001).

The lack of differences in ET-1 levels between the different groups suggests that ET-1 does not seem to play a role in this group of hypertensive women. Initially, it was thought that the size of the hypertensive group could be the reason for this discrepancy, however, the lack of differences obtained when the groups were re-divided into normotensive and hypertensive and then into lean and overweight/obese, proved this possibility to be unlikely. Additionally, the lack of associations of ET-1 with the decreased vascular function in the hypertensive group confirmed the absence of ET-1 in obesity-related hypertension in this group. One may speculate that genetic differences could be the reason for such a difference between Africans and African Americans. However, as mentioned, mere trends were reported here and needs confirmation by population-based studies.

The major finding of the study was the independent association that was obtained between leptin and blood pressure, which El-Gharbawy *et al.* (2002) failed to obtain in African Americans. The association of leptin with decreased C_W and increased PP in the hypertensive group also confirms leptin's association with deceased vascular function (Ciccone *et al.*, 2001; Singhal *et al.*, 2002) in this group. Furthermore, when leptin's associations with cardiovascular function of obese African and obese Caucasian women were compared, leptin showed a favourable association with vascular function in the obese Caucasian group, but not in the obese African group. The lack of favourable associations with vascular function in the obese African group, together with the afore-

mentioned findings, suggest that leptin predominantly exerts pathological influences on vascular function and blood pressure in obese African women.

6. CONCLUSIONS

The decreased vascular function and increased blood pressure observed in obesity-related hypertension in this group of African women is associated with an abdominal fat distribution and elevated leptin levels. ET-1 does not seem to be involved in obesity-related hypertension in this group. Although leptin also has favourable influences on vascular function, these influences seem to be more prominent in Caucasians, suggesting that leptin predominantly exerts pathological influences on vascular function and blood pressure in obese African women. These findings emphasise the importance of more elaborate investigations to be conducted on leptin in African women from South Africa.

7. RECOMMENDATIONS

The following recommendations are aimed at improving the cardiovascular health of obese African women:

- Abdominal subcutaneous fat may either be a marker of visceral fat or may in itself contribute to increased cardiovascular risk in Africans and should be reduced.

- Leptin is related to obesity-related hypertension and could possibly play a pro-atherogenic role in African women, decreasing C_W and increasing PP and SBP. Leptin levels should be decreased and/or countered at receptor level.

From this study certain recommendations arise which justifies and would improve further research:

- When studying obesity-related hypertension in Africans, group subdivisions should include blood pressure and not be based solely on BMI.

- To obtain clearer contrasts between groups, subject groups should be divided into lean normotensive, lean hypertensive, obese normotensive and obese hypertensive groups.

- Salt sensitivity and sympathetic activity have been reported to play a significant role in the development and/or maintenance of hypertension in Africans (Forrester, 2004) and should, therefore, always be assessed when studying this population group.

- Associations of sympathetic activity with leptin should be assessed in Africans.

- Differences in cardiovascular functioning between HIV-positive and negative patients exist and should be included as an exclusion criterion where ethically possible.

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