Leptin and cardiovascular function in African and Caucasian men and women: the SABPA study

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Preface

The *article format* as approved by the North-West University was used for this dissertation. Based on this format, the chapter division is as follows: Chapter 1 provides an introduction containing a short background and motivation. Chapter 2 is a literature overview of the topic with the aim, objectives and hypotheses provided at the end. Chapter 3 contains the manuscript for submission to *Hypertension Research* which includes the background, methods, results and interpretation of the study. Lastly, Chapter 4 consists of a summary of the main findings, and recommendations for future studies. Appropriate references are presented at the end of each chapter, according to the style of *Hypertension Research.*

Author contributions

The following researchers contributed to this study:

Ms C Pieterse

Involved in the collection of cardiovascular data using the Finometer- and Sphygmocor devices. The measurements of four participants with the above mentioned devices were obtained daily at the Metabolic Unit Research Facility for the duration of the study. Responsible for literature searches, design, planning and writing of the manuscript. Also, all statistical analyses as described in the methods section of Chapter 3.

Prof R Schutte

Supervisor

Involved in the collection of cardiovascular data, supervised the writing of the manuscript, made recommendations and provided statistical advice.

Prof AE Schutte

Co-supervisor

Responsible for the collection of cardiovascular data, provided advice for statistical analyses and guidance during the writing of the manuscript.

This is a statement from the co-authors confirming their individual role in the study and giving their permission that the article may form part of this dissertation.

Prof R Schutte **Prof AE Schutte**

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TITLE: Leptin and cardiovascular function in African and Caucasian men and women: the SABPA study

SUMMARY

Motivation

Hypertension and obesity are major risk factors for the development of cardiovascular disease. These risk factors also seem to be more common in the urban African population of sub-Saharan Africa. The literature indicates that elevated leptin levels may be the link between obesity and hypertension. Leptin elicits various detrimental cardiovascular actions and may thereby contribute to the development of hypertension and atherosclerosis. Therefore, the motivation for this study was to determine whether elevated leptin levels are associated with adverse cardiovascular health.

Aim

The aim of this study was to investigate circulating leptin levels and associations with cardiovascular function in urbanised Africans and Caucasians.

Methods

The study consisted of 409 African and Caucasian school teachers working in the Potchefstroom district in the North West Province of South Africa. Ambulatory blood pressure and carotid intima-media thickness measurements were obtained. A fasting blood sample was obtained and serum leptin levels were determined. Independent t-tests were performed to compare means between groups and the Chi-square test (χ^2) to compare proportions. We also tested the association between leptin and cardiovascular variables for interaction with ethnicity or gender by introducing appropriate interaction terms. Pearson's correlations, partial correlations and forward stepwise multiple regression analyses were performed to investigate associations and independent associations between cardiovascular measures and leptin.

Results and conclusion

Africans recorded higher body mass index ($P<0.001$) and leptin levels ($P<0.001$) than Caucasians. Furthermore, Africans also had higher blood pressure ($P<0.001$), carotid intima-media thickness ($P<0.01$) and cross-sectional wall area ($P<0.01$). However, we found no significant interactions with ethnicity or gender for the associations between the cardiovascular variables and leptin. Therefore, we focused on the total group. After adjustments were made for age, ethnicity and gender, positive associations of 24 h systolic blood pressure ($r=0.27$; $P<0.001$), 24 h diastolic blood pressure

(r=0.19; P<0.001), 24 h pulse pressure (r=0.25; P<0.001), carotid intima-media thickness (r=0.14; P=0.004) and cross-sectional wall area (r=0.18; P<0.001) with leptin were obtained. These findings were confirmed in multiple regression analyses after adjusting for significant covariates. When additionally adjusting for body mass index in multiple regression analyses, the association between cross-sectional wall area and leptin remained (R^2=0.439; β=0.121; P=0.019).

To conclude, circulating leptin levels showed a significant relationship with carotid cross-sectional wall area, independent of various confounders as well as ethnicity, gender and body mass index. Our findings therefore suggest that leptin may contribute to the development of atherosclerosis and thereby link obesity and cardiovascular disease.

Keywords: Atherosclerosis, blood pressure, cross-sectional wall area, ethnicity, leptin.

TITEL: Leptien en kardiovaskulêre funksie in manlike en vroulike Afrikane en Kaukasiërs: die SABPA-studie

OPSOMMING

Motivering

Hoë bloeddruk en obesiteit is belangrike risikofaktore vir die ontwikkeling van kardiovaskulêre siekte. Hierdie risikofaktore is ook algemener in die stedelike bevolking van Afrikane suid van die Sahara. Die literatuur dui aan dat verhoogde leptienvlakke die skakel tussen obesiteit en hoë bloeddruk kan wees. Leptien veroorsaak verskeie nadelige kardiovaskulêre reaksies en kan as gevolg daarvan bydra tot die ontwikkeling van hoë bloeddruk en aterosklerose. Die motivering vir hierdie studie was derhalwe om te bepaal of verhoogde leptienvlakke met swak kardiovaskulêre gesondheid geassosieer kan word.

Doel

Die doel van hierdie studie was om ‘n ondersoek te doen na sirkulerende leptienvlakke en assosiasies daarvan met kardiovaskulêre funksie in stedelike Afrikane en Kaukasiërs.

Metodes

Die studie het uit 409 Afrikane en Kaukasiërs bestaan wat in Suid-Afrika se Noordwesprovinsie as onderwysers in die Potchefstroom-distrik werk. Ambulatoriese bloeddrukmetings en carotis-intima-mediadiktemetings is verkry. ‘n Vastende bloedmonster is verkry en serumleptienvlakke is bepaal. Onafhanklike t-toetse is uitgevoer om gemiddeldes tussen groepe te vergelyk en die chi-kwadraattoets (χ^2) is gebruik om eweredighede te vergelyk. Die assosiasie tussen leptien en kardiovaskulêre veranderlikes is ook vir interaksie met etnisiteit of geslag getoets deur gepaste interaksieterme in te sluit. Pearson se korrelasies, parsiële korrelasies en voorwaartse, stapsgewyse meervoudige regressie-ontledings is uitgevoer om assosiasies en onafhanklike assosiasies tussen kardiovaskulêre metings en leptien te ondersoek.

Resultate en gevolgtrekking

Hoër liggaamsmassa-indeks ($P<0.001$) en leptienvlakke ($P<0.001$) is by Afrikane as by Kaukasiërs aangeteken. Verder het Afrikane ook hoër bloeddruk ($P<0.001$), carotis-intima-mediadikte ($P<0.01$) en deursnee-wandarea ($P<0.01$). Daar is egter geen beduidende interaksies met etnisiteit of geslag vir die assosiasies tussen die kardiovaskulêre veranderlikes en leptien nie. Gevolglik is daar op die totale groep gefokus. Nadat aanpassings vir ouderdom, etnisiteit en geslag gemaak is, is positiewe

assosiasies van 24 h sistoliese bloeddruk (r=0.27; P<0.001), 24 h diastoliese bloeddruk (r=0.19; P<0.001), 24 h polsdruk (r=0.25; P<0.001), carotis-intima-mediadikte (r=0.14; P=0.004) en deursnee-wandarea (r=0.18; P<0.001) met leptien verkry. Ná aanpassing vir beduidende kovariate is hierdie bevindinge in veelvuldige regressie-ontledings gestaaf. Met addisionele aanpassing vir liggaamsmassa-indeks in veelvuldige regressie-ontledings het die assosiasie tussen deursnee-wandarea en leptien (R^2=0.439; β=0.121; P=0.019) nog steeds bestaan.

Ten slotte: Sirkulerende leptienvlakke het ‘n beduidende verhouding met carotis-deursnee-wandarea aangedui, onafhanklik van verskeie strengelaars, sowel as etnisiteit, geslag en liggaamsmassa-indeks. Die bevindings dui dus daarop dat leptien tot die ontwikkeling van aterosklerose kan bydra en bring daardeur obesiteit en kardiovaskulêre siekte met mekaar in verband.

Sleutelwoorde: Aterosklerose, bloeddruk, deursnee-wandarea, etnisiteit, leptien.

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List of abbreviations

α-MSH:	α-melanocyte-stimulating hormone
ANP:	atrial natriuretic peptide
BMI:	body mass index
CO:	cardiac output
CRP:	C-reactive protein
DBP:	diastolic blood pressure
CIMT:	carotid intima-media thickness
CSWA:	cross-sectional wall area
HR:	heart rate
IL-6:	interleukin-6
FBG:	fibroblast growth factor
MC-4R:	melanocortin-4 receptor
MMP-2:	matrix metalloproteinase-2
NPY:	neuropeptide Y
POMC:	pro-opiomelanocortin
PP:	pulse pressure
ROS:	reactive oxygen species
SBP:	systolic blood pressure
SV:	stroke volume
TPR:	total peripheral resistance
VEGF:	vascular endothelial growth factor

CHAPTER 1

BACKGROUND AND MOTIVATION

Hypertension, a major risk factor for the development of stroke, heart and kidney disease, is a leading health concern in sub-Saharan Africa.[1] Populations of African descent seem more susceptible to hypertension, which occurs earlier in life than those of European descent.[2] In South Africa, the prevalence of hypertension varies with ethnicity, with urbanised Africans demonstrating the highest prevalence.[3,4] Dietary and lifestyle changes associated with urbanisation partly explain this susceptibility to hypertension[5] and contribute to the alarming rates of cardiovascular mortality.[3] It has been proposed that when members of this group are urbanised, the traditional rural diet is replaced with a high fat and low carbohydrate diet, contributing to weight gain.[6] This results in an increased prevalence of obesity which is a risk factor for numerous cardiovascular diseases, including hypertension.[7] Furthermore, obesity is reaching epidemic proportions in South Africa[5] and is more common in urban African women when compared to rural African women of sub-Saharan Africa.[4,8]

The mechanisms linking obesity and hypertension are not well understood, but possible underlying mechanisms include disturbed renal body fluid control, insulin resistance and endothelial dysfunction.[9,10] In addition, obesity is associated with the metabolic syndrome, a cluster of metabolic abnormalities which include dyslipidemia, insulin resistance, glucose intolerance and hypertension, which in turn contribute to atherogenesis.[11,12] The adipocytokine, leptin is secreted mainly by adipose tissue and has a variety of actions on multiple organ systems.[13] Current research suggests that elevated leptin levels may contribute to the pathogenesis of hypertension and atherosclerosis via multiple mechanisms.[14] It remains uncertain whether elevated leptin levels is directly involved in the progression of atherosclerosis in obese individuals.[14] Leptin increases sympathetic nerve activity to the kidney and adrenal gland, thereby increasing arterial pressure and heart rate.[10] In addition, leptin has been implicated in several cardiovascular diseases such as coronary heart disease and stroke,[9] however the link remains unresolved.

Previous studies on various ethnic populations demonstrate strong associations between hyperleptinemia, leptin resistance, the metabolic syndrome and cardiovascular complications.[15,16] Furthermore, growing evidence suggests that leptin alters numerous cardiovascular, autonomic and renal functions.[17]

Data with regards to leptin and cardiovascular function in black South Africans, especially African men, is limited. Therefore, this study will contribute to our understanding of leptin and how it relates to cardiovascular function in African and Caucasian men and women.

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CHAPTER 2
LITERATURE STUDY

1. Overview of leptin

White adipose tissue is the main source of leptin, a 16-kDa protein which primarily regulates energy expenditure and metabolism via specific receptors in the hypothalamus.[1] However, leptin is also synthesised in non-adipose tissue (skeletal muscle, placenta and stomach)[2] and may play a part in angiogenesis, glucose regulation, the immune and the reproductive system.[3] Leptin elicits a neuronal response upon binding to Ob-Rb receptors situated in the arcuate nucleus of the hypothalamus,[4] regulating appetite[1] by stimulating or inhibiting the secretion of neurotransmitters.[5] Leptin treatment has been shown to directly inhibit the release of neuropeptide Y,[6] a stimulus for increased food intake, corticosteroid and insulin secretion.[7,8] In addition, neuropeptide Y administration in animals lead to reduced sympathetic activity in the kidney and brown adipose tissue.[9] On the other hand, leptin stimulates sympathetic nerve activity in various tissues via multiple pathways.[4] Leptin binding to its receptor situated on pro-opiomelanocortin neurons induces α-melanocyte-stimulating hormone (α-MSH) release.[4] Subsequently, α-MSH activates the melanocortin-4 receptor which leads to increased sympathetic nerve activity in the kidney and brown adipose tissue.[4,10] Studies also show that leptin induced sympathetic activation in brown adipose tissue is dependent on corticotrophin-releasing factor.[4]

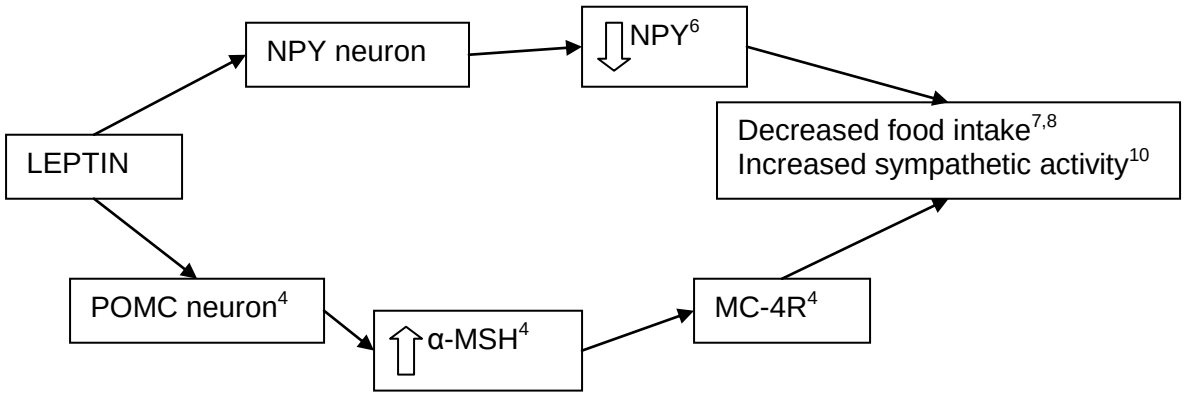

Figure 1. Leptin actions in the arcuate nucleus of the hypothalamus (NPY, neuropeptide Y; POMC, pro-opiomelanocortin; α-MSH, α-melanocyte-stimulating hormone; MC-4R, melanocortin-4 receptor). Figure adapted from Haynes.[4]

The concept of leptin resistance originated when obese human and animal model studies demonstrated a state of hyperleptinemia and not one of leptin deficiency.[11,12] Today it is believed that various scenarios may give rise to leptin resistance, such as leptin receptor or effector defects and inadequate leptin circulation or transport across the blood-brain barrier.[5]

Leptin is transported across the blood-brain barrier via a saturable system,[4] which is impaired or reduced in obese individuals.[5] In addition, leptin resistance appears to be selective. Those metabolic effects of leptin which stem from the arcuate nucleus are impaired, whereas sympathoexcitatory effects of leptin are maintained.[13] Its sympathetic vasomotor stimulatory action may take place in hypothalamic nuclei other than the arcuate nucleus.[13] Therefore, selective leptin resistance may be a possible mechanism for the development of obesity-related hypertension.[14]

2. Cardiovascular-related actions of leptin

2.1 Blood pressure

Apart from leptin's metabolic functions of appetite- and energy balance regulation,[15] its role in cardiovascular disease is poorly understood. It is suggested that leptin may be an important contributor to obesity-related hypertension.[16] Previous studies indicate that leptin may be a risk factor for cardiovascular events such as stroke and myocardial infarction.[15,17] This association between leptin and stroke was found in African American women, independent of age, obesity and hypertension.[15] The prevalence of hypertension and stroke is higher amongst urban Africans compared to Caucasians of sub-Saharan Africa.[18] Furthermore, obesity is associated with hypertension and the prevalence thereof is higher in African women than in African men and Caucasians.[18,19] An increase in tissue mass or adiposity subsequently raises the total blood volume, cardiac output, stroke volume and systemic vascular resistance and thereby contribute to the development of hypertension.[20] However, the mechanisms by which leptin may link obesity and hypertension are not entirely clear.

Previous studies have identified both beneficial and detrimental actions of leptin.[21] Selective leptin resistance and dose-dependent effects may partly contribute to these contradictory actions.[21] Selective leptin resistance observed in obese animal models and patients with hyperleptinemia, exhibited resistance to leptin's metabolic actions; however the stimulatory effect on the sympathetic nervous system remained intact.[1,14] Selective leptin resistance due to hyperleptinemia may therefore play a potentially pathologic role in the development of hypertension.[13] Worth mentioning is the fact that obese leptin deficient mice were not hypertensive and exhibited slightly reduced blood pressure compared to that of their lean counterparts.[22]

A recent study demonstrated a positive association between higher plasma leptin levels and hypertension, after adjustment for age, gender, body mass index, diabetes mellitus, cholesterol,

smoking, alcohol and C-reactive protein (CRP).[23] Also, it was established that intravenous leptin infusion activates sympathetic nervous system activity in the kidney and adrenal gland, which may disrupt cardiovascular homeostasis.[24] However, acutely infused leptin did not raise arterial blood pressure in normotensive and hypertensive rats.[25,26] Leptin's ability to induce vasodilation may override its ability to induce vasoconstriction via sympathetic overactivity, therefore resulting in little or no change in arterial blood pressure.[27,28] Furthermore, leptin receptors are present on the endothelium and promote vasodilation by stimulating nitric oxide production via endothelium dependent mechanisms[29] and nitric oxide independent mechanisms.[30] In rats, leptin treatment led to a dose-dependent increase in nitric oxide which in turn promoted smooth muscle cell relaxation.[31] Additionally, *in vitro* animal studies have shown that leptin promotes nitric oxide dependent vasodilation of coronary arterioles.[31] Interestingly, nitric oxide antagonism leads to leptin induced blood pressure elevation while blockage of the sympathetic nervous system causes leptin induced blood pressure reduction.[32] Therefore, leptin may contribute to a haemodynamic balance via its actions on the sympathetic nervous system through vasoconstriction and nitric oxide mediated vasodilation.[27]

2.2 Inflammation and oxidative stress

Multiple factors such as inflammation, matrix remodelling, endothelial dysfunction and smooth muscle cell proliferation contribute to the development and progression of atherosclerosis,[33] mainly in the larger elastic and muscular arteries.[34] It is known that leptin triggers migration, proliferation and hypertrophy of vascular smooth muscle cells.[35-37]

Angiogenesis is the process of new blood vessel formation[38] and is mediated by factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FBG) and nitric oxide.[39,40] The endothelium is a target for leptin and previous research has indicated that leptin, synergistically with FBG and VEGF, stimulated angiogenesis *in vivo.*[41] Additionally, research conducted on cultured endothelial cells demonstrated that physiological concentrations of leptin increased epithelial cell proliferation, which is a major event during angiogenesis.[42] The effect of leptin on endothelial cell proliferation was equal to that generated by VEGF; therefore leptin may be considered as an endothelial growth factor.[42]

Previous studies show that angiotensin II and endothelin-1 stimulate leptin synthesis in adipocytes.[43,44] Further, it has been proposed that vascular smooth muscle cell hypertrophy is evoked by exogenous leptin through mechanisms dependent on angiotensin II and endothelin-1.[45] Angiotensin II and endothelin-1 promote smooth muscle cell contraction and smooth muscle

cell hypertrophy which results in atherosclerosis.[34] It is known that vascular hypertrophy is an important event associated with hypertension[46] and leptin's contribution in this regard needs further investigation.[45]

Inflammation plays a key role in the progression of atherosclerosis and is characterised by raised levels of inflammatory markers such as CRP and interleukin-6 (IL-6).[47] CRP plays a major part in the accumulation of foam cells and monocytes in atherosclerotic plaque.[48] Its production takes place in the liver, primarily under IL-6 regulation.[49] Evidence confirms that leptin is also linked to inflammatory processes.[50] Leptin stimulates the synthesis of proinflammatory cytokines and decreases the production of anti-inflammatory cytokines, therefore indirectly contributing to endothelial dysfunction.[43] Interestingly, a strong positive association between leptin and CRP, independent of several factors such as age, gender, body mass index and waist-to-hip ratio, has previously been reported.[51] It is known that leptin can induce IL-6 production;[52] therefore it may indirectly upregulate the hepatic synthesis of CRP.[43] In addition, CRP has been shown to disrupt endothelium-dependent vasodilation, thereby contributing to hypertension.[53]

The relationship between leptin and reactive oxygen species (ROS) has been studied *in vitro* and it was found that leptin increases the generation of ROS in human umbilical vein endothelial cells.[54] In addition, leptin also increases the formation of ROS in bovine aortic endothelial cells in a dose-dependent manner.[55] Increasing evidence shows that both chronic and acute overproduction of ROS play an integral part in the onset of atherosclerosis and related cardiovascular diseases.[56] Leptin reduces antioxidant enzymes and promotes fatty acid oxidation, therefore contributing to increased formation of ROS.[55,57] Oxidative stress may indirectly increase atherogenic factors but also mediate direct damage to the endothelial and vascular smooth muscle cells.[13] It was demonstrated in hypertensive rat models that oxidative stress reduces nitric oxide bio-availability and leads to sodium retention and ultimately hypertension.[58]

2.3 Atherosclerosis

It is known that leptin promotes platelet aggregation and arterial thrombosis;[59] it also stimulates angiogenesis and the proliferation of vascular smooth muscle cells.[60] Therefore, leptin possesses atherogenic and growth promoting properties which may contribute to increased cardiovascular disease risk.[61]

Leptin promotes the expression of matrix metalloproteinase (MMP)-2 in cultured human aortic smooth muscle cells and increases the proliferation of these cells.[35] MMP-2 is highly expressed in atherosclerotic lesions and leads to the migration of smooth muscle cells toward the intima.[62] The proliferation of rabbit vascular smooth muscle cells *in vitro* was reduced when treated with MMP inhibitors.[63] Additionally, MMPs also contribute to proliferation of cells by participating in the activation of growth factors.[64]

The measurement of carotid intima-media thickness (CIMT) is an indicative marker of general atherosclerosis and could identify individuals that are at risk for coronary heart disease.[65] CIMT may predict cardiovascular events such as stroke and myocardial infarction,[66] and is associated with coronary artery disease.[67] The link between obesity and carotid atherosclerosis remains largely unknown.[68] However, it is suggested that metabolic factors such as insulin resistance and low adiponectin levels may contribute to early atherosclerosis.[69] Studies regarding the association of CIMT with leptin and adiponectin demonstrate contradictory results. A significant correlation between leptin:adiponectin ratio and CIMT has been found independent of body mass index.[70] In addition, when plasma leptin levels were examined alone a significant positive association with CIMT was found.[70,71] On the other hand, a recent study did not find associations of CIMT with the leptin:adiponectin ratio and leptin.[72] The impact of leptin on atherosclerosis remains unresolved, but experiments conducted on apolipoprotein E-deficient mice indicate that leptin promotes atherosclerosis and thrombosis.[73]

Increased total blood volume and stroke volume which accompany increased body size may also result in increased luminal diameter and subsequently lead to increased CIMT to normalise greater shear stress.[68] Furthermore, it has been established that men have a higher CIMT than women and that Africans have a higher CIMT when compared to Caucasians.[65] A recent study also demonstrated that Africans, after adjustment for traditional risk factors, had a higher CIMT than South Asians.[74]

2.4 Arterial stiffness

Measures of arterial distensibility reflect the elastic properties of an artery and may be an indicative marker of coronary heart disease risk.[75,76] Evidence suggests that elevated leptin levels are associated with decreased arterial distensibility, possibly due to mechanisms underlying the atherosclerotic process.[60] Leptin receptors are present on vascular smooth muscle cells and play a part in regulating vascular compliance, independent of body mass.[77] Hyperleptinemia is associated with vascular calcification[78] and poor vascular compliance,[60]

possibly due to leptin resistance rather than elevated leptin levels.[79] A previous study determined that leptin was negatively associated with arterial compliance in obese/overweight hypertensive African women.[80] Furthermore, it has been suggested that leptin is a better predictor of vascular compliance than commonly used factors such as fasting insulin and CRP.[81] Animal studies have shown that when wild-type mice were treated with a leptin antagonist, increased aortic stiffness resulted; however, leptin administration to leptin-deficient mice reduced vascular stiffness.[77]

2.5 Pro-thrombotic actions

Leptin promotes arterial wall calcification and thrombosis by promoting platelet aggregation.[82,83] Accumulating evidence demonstrates that leptin may be a crucial regulator of arterial thrombosis *in vivo.*[84] The thrombotic response to vascular injury is reduced in leptin-deficient mice compared to lean wild-type mice, while inhibition of endogenous leptin in lean mice also decreased the thrombotic response.[33,84,85] It was suggested that leptin-associated tumour necrosis factor-α and IL-6 secretion may contribute to increased thrombus formation which in turn promotes endothelial dysfunction and coronary heart disease.[83] In addition, leptin slightly reduces the expression of the anti-thrombotic protein, thrombomodulin, in cultured endothelial cells isolated from the umbilical vein.[86] Further, a previous study showed that thrombogenic factors, such as fibrinogen and the von Willebrand factor, are significantly correlated with leptin.[87,88]

2.6 Leptin and the heart

Leptin production also takes place in the heart and cardiac leptin receptors also exist.[89] Resultantly, leptin may elicit direct physiological effects on cardiomyocytes.[90] The possible link between hyperleptinemia and coronary artery disease remains unresolved.[90] However, leptin levels were found to be twofold higher after myocardial infarction.[91] Also, an independent positive association between leptin levels and heart rate was demonstrated in heart transplant patients with sympathetic denervation; therefore the increased heart rate was not due to leptin's sympathetic stimulatory effect.[89,92] While tachycardia may provide short-term benefits by improving cardiac output, chronic tachycardia may lead to cardiac hypertrophy and result in heart failure.[92]

In vitro studies indicated that leptin induced neonatal rat cardiomyocyte hypertrophy via the generation of endothelin-1 and the production of ROS.[93] Also, in rat ventricular myocytes, it was demonstrated that acute leptin infusion enhances nitric oxide activity and decreases cardiac

contractility.[94] This effect may be due to leptin's ability to promote nitric oxide synthesis, thereby decreasing intracellular calcium and contractility.[95]

It was found that leptin deficiency or resistance led to enhanced cardiomyocyte apoptosis, which could contribute to the development of heart failure.[96] Leptin may therefore also provide cardiac protection. Mice receiving leptin treatment after myocardial infarction had a smaller infarct size compared to mice who did not receive leptin treatment.[95] Studies also showed that leptin administration protected rat cardiomyocytes from lipotoxicity, which commonly occurs during states of leptin resistance or leptin deficiency.[97,98]

In addition, recent evidence suggests that leptin may reduce plasma levels of atrial natriuretic peptide (ANP).[99] ANP regulates blood pressure by promoting diuresis, natriuresis and vasodilation.[100] Sprague-Dawley rats infused with leptin demonstrated reduced ANP levels and raised blood pressure.[99] This result was possibly due to leptin's effect of stimulating nitric oxide release, which in turn inhibits the secretion of ANP.[101,102]

2.7 Leptin and insulin

Leptin resistance or leptin deficiency may affect the pancreas by inducing cellular lipotoxicity which in turn may contribute to insulin resistance or β-cell dysfunction due to triglyceride accumulation in pancreatic tissue.[13] Leptin promotes fatty acid oxidation, thereby counteracting the antioxidant and lipogenic effects of insulin.[1] In addition, leptin treatment has been shown to reduce intracellular triglycerides and increase glucose-stimulated insulin secretion.[103,104] On the other hand, studies on lean Wistar rats indicate that leptin treatment reduces insulin levels without affecting plasma glucose levels.[105] Leptin may also increase insulin sensitivity without altering endogenous insulin secretion, which in turn may provide depressor effects.[106] Leptin inhibits the pancreatic β-cell synthesis and secretion of insulin, whereas leptin secretion from adipose tissue is promoted by insulin.[1] A previous study demonstrated that hyperinsulinemia promotes sodium and water reabsorption by tubular cells of the kidney, thereby promoting volume-dependent hypertension.[107] Another study indicated that increases in physiological insulin levels resulted in sympathetic stimulation but did not raise arterial pressure.[105] This may be explained by insulin's ability to induce vasodilation.[108] In addition, correlations between hyperinsulinemia, insulin resistance and hypertension exist.[109]

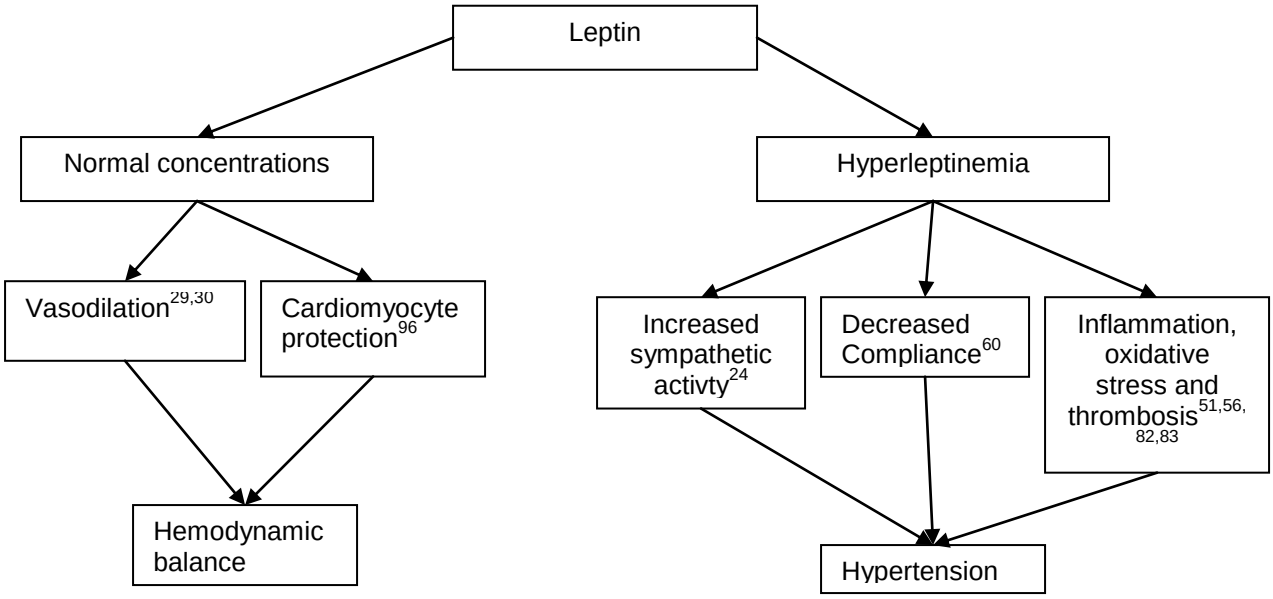

Figure 2. Cardiovascular actions of leptin. Figure adapted from Correia.[13]

3. Determinants of leptin levels

3.1 Adipose tissue

Serum leptin levels range from 10 ng/ml to 40 ng/ml in lean to obese individuals.[110] Leptin is primarily secreted by white adipose tissue[13] and therefore a significant relationship between leptin and measures of obesity such as body mass index and total body fat content exists.[111] Another determinant of leptin levels may be the differential distribution of adipose tissue in either visceral or subcutaneous compartments. *In vitro* findings demonstrate that subcutaneous adipocytes release approximately twice the amount of leptin that visceral adipocytes do.[112] Not surprisingly then, weight loss results in a reduction in leptin levels.[113] Apart from adipose tissue, leptin secretion may also be enhanced by other factors such as increased estrogens, androgens, insulin, glucocorticoids and free fatty acids.[114]

3.2 Lifestyle factors

Noticeable differences in leptin levels, for a certain body mass index or total body fat content exist; therefore factors other than body composition may also affect leptin levels.[115] Various behavioural factors such as alcohol intake, smoking and exercise could also influence these levels.[110] A modest association between physical activity and leptin levels has been reported in men, where the least active individuals had higher leptin levels.[110] A previous study also demonstrated that nonsmokers have higher leptin levels than smokers with a similar body mass index.[116] Furthermore, leptin levels were reported to be lower amongst heavy drinkers than

those who abstained from alcohol use.[110] This difference in leptin levels may possibly due to an inhibitory effect of alcohol on adipocytes.[117]

3.3 Ethnicity and gender

Only a few studies exist identifying ethnic differences in leptin concentrations.[118] It has been shown that African Americans have more subcutaneous adipose tissue and less visceral adipose tissue than their Caucasian counterparts.[119] Additionally, a previous study indicated that leptin is significantly higher in lean black South Africans compared to lean Caucasian women.[120] Obese African and obese Caucasian women however recorded similar leptin levels.[120] Another study reported contradictory results, where African American women had lower leptin levels than Caucasian women within the same adiposity range.[121] Furthermore, it was found that Asian-Indian men have higher leptin levels than Caucasian men after adjustment for body mass index.[122] Again, factors other than body fat may account for ethnic differences in leptin levels[118] and could contribute to different cardiovascular outcomes.[112]

Various studies regarding gender and leptin levels confirm that women have higher leptin concentrations than men.[110,111,118] These findings may be attributed to the presence of more subcutaneous fat in women or gender-related hormonal differences.[123]

4. Leptin treatment and weight loss

The use of leptin as a potential anti-obesity hormone has received much attention since its discovery. A study involving lean and obese individuals receiving exogenous leptin demonstrate successful reduction in weight- and fat loss.[124] The aforementioned study also noted that there was no significant detrimental effects on the major organ systems. Almost all obese individuals display a state of leptin resistance.[125,126] Therefore, it is questionable whether elevating leptin levels further by injecting exogenous leptin, is an effective weight loss treatment. In children with congenital leptin deficiency, leptin therapy has been shown to have beneficial effects.[127,128] But, in light of leptin's potential atherogenic, thrombotic and sympathetic nervous system stimulatory action,[129,130,131] future research is warranted to determine whether leptin therapy is a safe and effective anti-obesity treatment.

5. Aim, objectives and hypotheses

The aim of this study is to investigate leptin levels and the associations thereof with cardiovascular function in urbanised Africans and Caucasians.

The objectives are:

- to determine whether ethnic and gender differences exist in leptin levels of African and Caucasian men and women; and
- to investigate associations of leptin with markers of cardiovascular function in African and Caucasian men and women.

With regards to the literature, the hypotheses are:

- Women have higher leptin levels than men.
- Africans have higher leptin levels compared to their Caucasian counterparts.
- Africans have higher 24 h blood pressure and carotid intima-media thickness than Caucasians.
- Serum leptin is positively associated with 24 h blood pressure and carotid intima-media thickness, with stronger associations found in Africans than Caucasians.

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CHAPTER 3

LEPTIN AND CARDIOVASCULAR FUNCTION IN AFRICAN AND CAUCASIAN MEN AND WOMEN: THE SABPA STUDY

Summary of the instructions for authors: *Hypertension Research*

1. Title page with informative title, authors, running title and contact details of the corresponding author.

2. Abstract of not more than 250 words. It should outline the purpose of the study, the basic procedures and the most important conclusions. Three to five keywords should be given in alphabetical order below the abstract.

3. Introduction. Short, clear account of the background and reasons for undertaking the study

4. Methods. This section should contain sufficient detail so that all experimental procedures can be repeated. This section may be divided into subheadings to assist the reader. Names of products and manufacturers should be included.

5. Results. The results section should be concise and follow a logical sequence. This section may be divided into subheadings to assist the reader.

6. Discussion. Do not recapitulate the results, but discuss their significance against the background of existing knowledge, and identify clearly those aspects that are novel. The final paragraph should highlight the main conclusion(s).

7. Acknowledgements. These should be brief.

8. References. Each reference should be numbered individually. Example: Addo J, Smeeth L, Leon DA. Hypertension In Sub-Saharan Africa: A Systematic Review. *Hypertension* 2007; 50:1012-1018.

9. Tables. These should be labelled sequentially as Table 1, Table 2, etc. Tables should have a brief footnote that identifies all abbreviations used.

10. Figures. These should be labelled sequentially as Figure 1, Figure 2, etc.

Carotid cross-sectional wall area is significantly associated with serum leptin levels, independent of body mass index: the SABPA study

Short Title: Leptin and cardiovascular function in Africans and Caucasians

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Abstract

Hypertension and obesity are serious health burdens in sub-Saharan Africa and urbanised Africans seem to be more susceptible to the development of these diseases than Caucasians. Current research suggests that leptin may be an important contributor to the development of hypertension and atherosclerosis. The aim of this study was to investigate leptin levels and associations with cardiovascular function in urbanised Africans and Caucasians. Serum leptin, ambulatory blood pressure and carotid intima-media thickness were measured, and the cross-sectional wall area calculated. Results showed that Africans had higher leptin levels ($P<0.001$), ambulatory blood pressure ($P<0.001$), carotid intima-media thickness ($P<0.01$) and cross-sectional wall area ($P<0.01$) than Caucasians. Because we found no interaction with ethnicity and gender for the association between leptin and the cardiovascular variables, we focused mainly on the total group of Africans and Caucasians. In single, partial and multiple regression analyses, positive associations between ambulatory systolic blood pressure ($\beta=0.261$; $P<0.001$), diastolic blood pressure ($\beta=0.144$; $P=0.012$), pulse pressure ($\beta=0.335$; $P<0.001$) and cross-sectional wall area ($\beta=0.121$; $P=0,019$) with leptin were observed. Even after adjusting for body mass index, the association obtained between cross-sectional wall area ($\beta=0.121$; $P=0.019$) and leptin remained. Our findings therefore suggests that leptin may contribute to the development of atherosclerosis, independent of body mass index.

Keywords: Atherosclerosis, blood pressure, cross-sectional wall area, ethnicity, leptin.

Introduction

Hypertension is a serious public health concern worldwide, and its prevalence in sub-Saharan Africa is rapidly increasing.[1] The recent prevalence rates in sub-Saharan Africa are proposed to be 16.2%, and to be higher in urban than rural populations.[2] Urbanisation may therefore explain the observed variance in hypertension rates in sub-Saharan Africa.[3] With urbanisation there is a subsequent increase in hypertension and obesity rates - both contributors to higher cardiovascular disease risk.[4] The adipocytokine, leptin, is believed to be a possible link between obesity and cardiovascular disease.[5]

Subcutaneous adipose tissue is the primary source of leptin and the secretion of the latter correlates directly with body fat mass.[6] Leptin has both beneficial and detrimental effects on the cardiovascular system. On the one hand it induces vasodilation by promoting nitric oxide release in the endothelium[7] but, on the other, leptin increases sympathetic nerve activity[6] and promotes the release of vasoconstrictive substances[8] and damaging reactive oxygen species[9] which could contribute to obesity-related hypertension. As seen in the obese, a hyperleptinemic state also causes endothelial dysfunction and the proliferation of vascular smooth muscle cells and thereby promotes atherosclerosis.[10] It therefore comes as no surprise that leptin is an independent predictor of cardiovascular events such as myocardial infarction, and ischemic and non-ischemic stroke.[11] These findings were exclusively from Caucasian population groups or *in vitro* and *in vivo* studies.

To the best of the authors' knowledge, no studies regarding the associations of 24 h blood pressure and carotid intima-media thickness with leptin have been undertaken with respect to Africans. The aim of this study was therefore to compare leptin levels and their associations with cardiovascular function in urbanised African and Caucasian school teachers.

Methods

Study population

This study forms part of the SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans) study, which included 409 African and Caucasian school teachers working in the Potchefstroom district in the North West Province of South Africa. The reason for the selection of this target population was to obtain a homogenous sample of participants from a similar socioeconomic class. Participants between the ages of 25 and 60 years were included in this study. The exclusion criteria were an oral temperature above 37°C, psychotropic substance dependence or abuse, regular blood donors and individuals vaccinated in the past three months. Of the 409 subjects, we excluded one African and one Caucasian male from all analyses due to missing data. Participants received detailed information about the procedures and objectives of the study prior to their recruitment. Participants requesting conveyance of information in their home language were assisted. All participants signed an informed consent form. The study complied with all applicable requirements and international regulations, including the Helsinki declaration of 1975 (as revised in 2008) for investigation of human participants. The study was approved by the Ethics Review Board of the North-West University (Potchefstroom Campus).

Cardiovascular measurements

Ambulatory blood pressure measurements (ABPM) were conducted during the working week. At approximately 08h00, an ABPM apparatus (Meditech CE120® Cardiotens; Meditech, Budapest, Hungary) and two-lead electrocardiogram (ECG) apparatus were attached on the participant's non-dominant arm at their workplace. The ABPM apparatus was programmed to measure blood pressure at 30 minute intervals during the day (08h00-22h00) and every hour during night time. Participants received ambulatory diary cards and were requested to indicate abnormalities such as nausea, headache or stress experienced during their normal daily activities. At 16h30 participants were transported to the North-West University and admitted to the Metabolic Unit Research Facility. This facility consists of 10 bedrooms, two bathrooms, a living room and a kitchen. Participants received a standardised dinner and at 20h30 they received their last beverage (coffee/tea and two biscuits). They were then allowed to relax by reading, watching television or social interaction and were encouraged to go to bed at 22h00. Participants were requested to refrain from alcohol consumption, caffeine consumption and exercise. At 06h00 the ABPM apparatus was removed and subsequent measurements commenced. Electrocardiogram and 24 h blood pressure data were downloaded onto a database using the CardioVisions 1.9.0 Personal Edition (Meditech, Budapest, Hungary). If less than 75% of the ABPM recordings for a particular participant was successful, the measurement was repeated the next day. The validated[12,13] Finometer device (FMS, Finapres

Measurement Systems, Amsterdam, The Netherlands) was connected, and a 5-min continuous measurement of resting cardiovascular parameters was carried out. Carotid intima-media thickness (CIMT) measurements were obtained using a SonoSite Micromaxx ultrasound system (SonoSite, Bothell, WA) and a 6-13 MHz linear array transducer. Images from at least two optimal angles of the left and right common carotid artery were obtained. Following previous prescribed protocols,[14] these segments were imaged and measured. The images were digitised and imported into the Artery Measurement Systems automated software[15,16] for dedicated analyses of CIMT. A maximal 10 mm segment with good image quality was chosen for analysis. The program automatically identifies the borders of the intima-media of the near and far wall, and the inner diameter of the vessel, and calculates the CIMT and diameter from around 100 discrete measurements through the 10 mm segment. This automated analysis was capable of being manually corrected if not found appropriate on visual inspection. Far wall measurements were used for the present study. Intraobserver variability for the far wall was 0.04 mm between two measurements made four weeks apart on 10 subjects. We also calculated the cross-sectional wall area (CSWA) to confirm structural and not functional changes in luminal diameter as follows: $CSWA = \pi(d/2 + CIMT)^2 - \pi(d/2)^2$, where d denotes luminal diameter.

Anthropometric measurements

Height (stature) and weight of participants were measured while being in their underwear using calibrated instruments (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465; Invicta, London, UK). Subsequently, the body mass index (BMI) was calculated for each participant. All measurements were taken in triplicate using standard methods.[17]

Biochemical measurements

After the cardiovascular and anthropometric measurements were done, a registered nurse obtained a fasting blood sample with a sterile winged infusion set from the antebrachial vein branches. EDTA whole blood and serum were stored at –80 °C. Serum glucose was determined using a timed-end-point method (Unicel DXC 800, Beckman and Coulter, Germany). Fasting serum samples for total cholesterol and high-sensitivity C-reactive protein were analysed using the sequential multiple analyser computer (Konelab 20i; Thermo Scientific, Vantaa, Finland). Serum reactive oxygen species (ROS) were determined by an improved assay system based on the principle of the derivatives of reactive oxygen metabolites test, which is recognized as an efficient method for evaluating oxidative stress in the body. The Bio-Tek FL600 Microplate Fluorescence Reader (Bio-Tek, Instruments, Inc., Highland Park, Winooski, VT, USA) was used to measure ROS levels, where 1.0 mg/l H_2O_2 represents one unit of ROS.[18] For alcohol intake, gamma-glutamyl transferase

levels were measured with the UniCel DxC 800 analyser (Beckman and Coulter, Germany) on an enzyme rate method.[19] Serum leptin levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Quantikine, R&D Systems, MN, USA). HIV testing was performed with First Response HIV Card Test 1-2.0 (PMC Medical, India Pvt Ltd) and confirmed with Pareekshak HIV Triline (UCB Pharma).

Statistical Analyses

For database management and statistical analyses, we used Statistica software version 10.0 (Statsoft, Inc., Tulsa, OK, 2010). The distribution of serum leptin, serum glucose, high-sensitivity C-reactive protein and gamma-glutamyl transferase were normalised by logarithmic transformation. The central tendency and spread of these variables were represented by the geometric mean and the 5^{th} and 95^{th} percentile intervals. Independent t-tests were done to compare means between groups and the Chi-square test (χ^2) to compare proportions. Mean values of leptin were plotted by pentiles of the cardiovascular variables to ensure that linear correlation techniques were appropriate. Pearson's correlations were determined as well as partial correlations after adjustment for ethnicity, gender and age. We also tested interactions with ethnicity and gender for the association between leptin and cardiovascular variables by introducing appropriate interaction terms. Forward stepwise multiple regression analyses were performed in the total group to investigate independent associations of 24 h SBP, 24 h DBP, 24 h PP, CIMT and CSWA with leptin while adjusting for significant covariates (ethnicity, gender, age, 24 h mean arterial pressure, total cholesterol, serum glucose, C-reactive protein, smoking, gamma-glutamyl transferase, HIV-infection and anti-hypertensive medication).

Results

Characteristics of participants

Table 1 lists the characteristics of the total group (n=407), as well as the Africans (n=199) and Caucasians (n=208) seperately. Africans were shorter than Caucasians, but body mass and waist circumference did not differ. Both the body mass index (P<0.001) and leptin levels (P<0.001) of Africans were higher than Caucasians. In addition, Africans accounted for 36% of the overweight or obese individuals in the total group, whereas 32% were Caucasians (P=0.026). Furthermore, Africans had lower total cholesterol (P<0.001), higher C-reactive protein (P<0.001) and reactive oxygen species (P=0.049), blood pressure (P<0.001), CIMT (P<0.01) and CSWA (P<0.01) than Caucasians. In both Africans and Caucasians, women had significantly higher leptin levels than men (P<0.001).

Table 1 Characteristics of study population.

	Total group (n=407)	Africans (n=199)	Caucasians (n=208)	P
Age, years	44.7 ± 9.5	44.4 ± 7.9	44.9 ± 10.8	0.54
Women, n (%)	207 (50.9)	99 (49.7)	108 (51.9)	0.66
Stature, m	1.69 ± 0.10	1.65 ± 0.09	1.74 ± 0.10	<0.001
Body mass, kg	82.7 ± 19.9	81.5 ± 18.4	84.0 ± 21.1	0.21
Waist circumference, cm	93.4 ± 15.7	93.7 ± 15.4	93.1 ± 16.1	0.68
Body mass index, kg/m^2	28.9 ± 6.58	30.2 ± 6.99	27.6 ± 5.93	< 0.001
Biochemical measurements				
Leptin, ng/ml	17.6 (3.06 - 85.6)	24.2 (3.11 - 95.8)	13.0 (2.62 - 55.8)	< 0.001
Total cholesterol, mmol/l	5.09 ± 1.32	4.60 ± 1.19	5.54 ± 1.27	< 0.001
Serum glucose, mmol/l	5.52 (4.31 - 7.33)	5.41 (4.03 - 10.4)	5.62 (4.70 - 6.89)	0.059
C-reactive protein, mg/l	2.95 (0.89 - 23.0)	4.46 (0.64 - 33.0)	2.03 (0.98 - 9.00)	< 0.001
ROS, units	93.1 ± 27.9	95.9 ± 26.4	90.4 ± 29.0	0.049
Cardiovascular measurements				
24 h SBP, mmHg	129 ± 14.9	133 ± 16.2	124 ± 12.0	< 0.001
24 h DBP, mmHg	79.9 ± 10.0	83.5 ± 10.7	76.6 ± 8.05	< 0.001
24 h PP, mmHg	48.6 ± 8.34	49.7 ± 9.09	47.5 ± 7.40	< 0.01
24 h Heart rate, bpm	76.6 ± 10.8	79.7 ± 10.8	73.8 ± 10.1	< 0.001
Cardiac output, l/min	6.64 ± 1.94	6.86 ± 1.91	6.44 ± 1.94	0.028
Stroke volume, ml	99.6 ± 25.9	101.6 ± 27.5	97.8 ± 24.3	0.14
Total peripheral resistance, mmHg/ml/s	1.03 ± 0.46	1.01 ± 0.38	1.04 ± 0.52	0.56
CIMT (far wall), mm	0.67 ± 0.15	0.69 ± 0.14	0.64 ± 0.14	< 0.01
CSWA, mm^2	13.8 ± 4.0	14.3 ± 4.1	13.2 ± 3.8	< 0.01
Lifestyle				
Physical activity, kcal	2907 ± 1287	2687 ± 791.6	3117 ± 1599	< 0.001
Smoking, n (%)	63.0 (15.5)	34.0 (17.1)	29.0 (14.0)	0.39
Gamma-glutamyl transferase, U/L	30.0 (8.00 - 130)	47.6 (20.1 - 183)	19.4 (6.99 - 76.0)	< 0.001
Antihypertensive medication, n (%)	61.0 (14.9)	43.0 (21.6)	18 (8.7)	< 0.001
Antidiabetic medication, n (%)	12 (2.9)	10 (5.0)	2 (1.0)	0.015

Values are arithmetic mean ± s.d., geometric mean (5th - 95th percentile interval), or number of participants (%).

P denotes difference between Africans and Caucasians.

Abbreviations: ROS, reactive oxygen species; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; CIMT, carotid intima-media thickness; CSWA, cross-sectional wall area.

Unadjusted analyses

There were no significant interactions with ethnicity or gender for the associations between the cardiovascular variables and leptin. Therefore our main focus is on the total group. However, for transparency, associations for the separate ethnic and gender groups are also shown. In single regression analyses (Table 2), all cardiovascular variables and ROS showed significant positive associations with leptin in the total group, except for age, 24 h DBP, CIMT and CSWA. In addition, when the separate ethnic and gender groups were investigated we found mixed results confirming the lack of interaction obtained with gender and ethnicity. However, the association in African men were prominent where positive associations of all cardiovascular variables including CIMT and CSWA with leptin were found.

Table 2 Unadjusted associations of leptin with age, body mass index, cardiovascular variables and reactive oxygen species.

	Total group	African men	African women	Caucasian men	Caucasian women
Age, years	r = 0.06; P = 0.21	r = 0.20; P = 0.043	r = 0.17; P = 0.086	r = -0.008; P = 0.94	r = -0.09; P = 0.34
BMI, kg/m^2	r = 0.62; P < 0.01	r = 0.82; P < 0.01	r = 0.70; P < 0.001	r = 0.76; P < 0.01	r = 0.69; < 0.001
24 h SBP, mmHg	r = 0.11; P = 0.028	r = 0.35; P < 0.001	r = 0.13; P = 0.20	r = 0.28; P = 0.005	r = 0.39; P < 0.001
24 h DBP, mmHg	r = 0.005; P = 0.91	r = 0.28; P = 0.005	r = -0.05; P = 0.61	r = 0.30; P = 0.002	r = 0.28; P = 0.004
24 h PP, mmHg	r = 0.19; P < 0.001	r = 31; P = 0.002	r = 0.25; P = 0.014	r = 0.08; P = 0.44	r = 0.36; P < 0.001
24 h HR, bpm	r = 0.32; P < 0.001	r = 0.26; P = 0.024	r = 0.02; P = 0.84	r = 0.41; P < 0.001	r = 0.37; P < 0.001
CO, l/min	r = 0.35; P < 0.001	r = 0.37; P < 0.001	r = 0.49; P < 0.001	r = 0.44; P < 0.001	r = 0.51; P < 0.001
SV, ml	r = 0.21; P < 0.001	r = 0.21; P = 0.044	r = 0.49; P < 0.001	r = 0.24; P = 0.015	r = 0.44; P < 0.001
TPR, mmHg/ml/s	r = -0.23; P < 0.001	r = -0.29; P = 0.004	r = -0.35; P = 0.001	r = -0.12; P = 0.25	r = -0.46; P < 0.001
CIMT (far wall), mm	r = 0.05; P = 0.31	r = 0.20; P = 0.046	r = 0.13; P = 0.21	r = 0.09; P = 0.38	r = 0.12; P = 0.21
CSWA, mm^2	r = -0.04; P = 0.45	r = 0.24; P = 0.019	r = 0.11; P = 0.26	r = 0.16; P = 0.11	r = 0.14; P = 0.14
ROS, units	r = 0.45; P < 0.01	r = 0.13; P = 0.18	r = 0.31; P = 0.002	r = 0.41; P < 0.001	r = 0.25; P = 0.009

Abbreviations: BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HR, heart rate; CO, cardiac output; SV, stroke volume; TPR, total peripheral resistance; CIMT, carotid intima-media thickness; CSWA, cross-sectional wall area; ROS, reactive oxygen species.

Adjusted analyses

After adjusting for ethnicity, gender and age in the total group (Table 3), we noticed positive associations of all cardiovascular variables and ROS (P<0.01) with leptin. After adjusting for age in the separate ethnic and gender groups, all of the previous unadjusted associations remained. However, the positive correlations of CIMT and CSWA with leptin in African men disappeared, but positive associations were obtained between CSWA and leptin in Caucasian men and both CIMT and CSWA with leptin in Caucasian women.

Table 3 Partial correlation coefficients of leptin with body mass index, cardiovascular variables and reactive oxygen species, adjusted for age

	Total group*	African men	African women	Caucasian men	Caucasian women
BMI, kg/m^2	r = 0.72; P < 0.01	r = 0.82; P < 0.01	r = 0.71; P < 0.001	r = 0.76; P < 0.01	r = 0.70; P < 0.001
24 h SBP, mmHg	r = 0.27; P < 0.001	r = 0.31; P = 0.002	r = 0.099; P = 0.33	r = 0.28; P = 0.006	r = 0.43; P < 0.001
24 h DBP, mmHg	r = 0.19; P < 0.001	r = 0.24; P = 0.015	r = -0.077; P = 0.45	r = 0.31; P = 0.002	r = 0.29; P = 0.002
24 h PP, mmHg	r = 0.25; P < 0.001	r = 0.27; P = 0.006	r = 0.22; P = 0.029	r = 0.08; P = 0.43	r = 0.39; P < 0.001
24 h HR, bpm	r = 0.26; P < 0.001	r = 0.20; P = 0.047	r = 0.03; P = 0.79	r = 0.41; P < 0.001	r = 0.36; P < 0.001
CO, l/min	r = 0.45; P < 0.01	r = 0.36; P < 0.001	r = 0.51; P < 0.001	r = 0.44; P < 0.001	r = 0.50; P < 0.001
SV, ml	r = 0.34; P < 0.001	r = 0.19; P = 0.053	r = 0.50; P < 0.001	r = 0.24; P = 0.015	r = 0.44; P < 0.001
TPR, mmHg/ml/s	r = -0.25; P < 0.001	r = -0.32; P = 0.002	r = -0.38; P < 0.001	r = -0.12; P = 0.25	r = -0.47; P < 0.001
CIMT (far wall), mm	r = 0.14; P = 0.004	r = 0.11; P = 0.26	r = 0.05; P = 0.62	r = 0.11; P = 0.28	r = 0.21; P = 0.027
CSWA, mm^2	r = 0.18; P < 0.001	r = 0.15; P = 0.12	r = 0.038; P = 0.71	r = 0.20; P = 0.049	r = 0.22; P = 0.020
ROS, units	r = 0.23; P < 0.001	r = 0.07; P = 0.49	r = 0.31; P = 0.002	r = 0.41; P < 0.001	r = 0.25; P = 0.010

*; Additionally adjusted for ethnicity and gender

Abbreviations: BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HR, heart rate; CO, cardiac output; SV, stroke volume; TPR, total peripheral resistance; CIMT, carotid intima-media thickness; CSWA, cross-sectional wall area; ROS, reactive oxygen species

With regard to measures of arterial structure and function, the associations obtained in the total group (Table 3) were confirmed in exploratory analyses (Figure 1) where 24 h SBP, 24 h PP and CSWA were plotted by pentiles of leptin, with adjustment applied for ethnicity, gender and age. The significant positive associations of 24 h SBP (P for trend <0.001), 24 h PP (P for trend <0.001) and CSWA (P for trend, 0.007) with leptin were again evident in the total group.

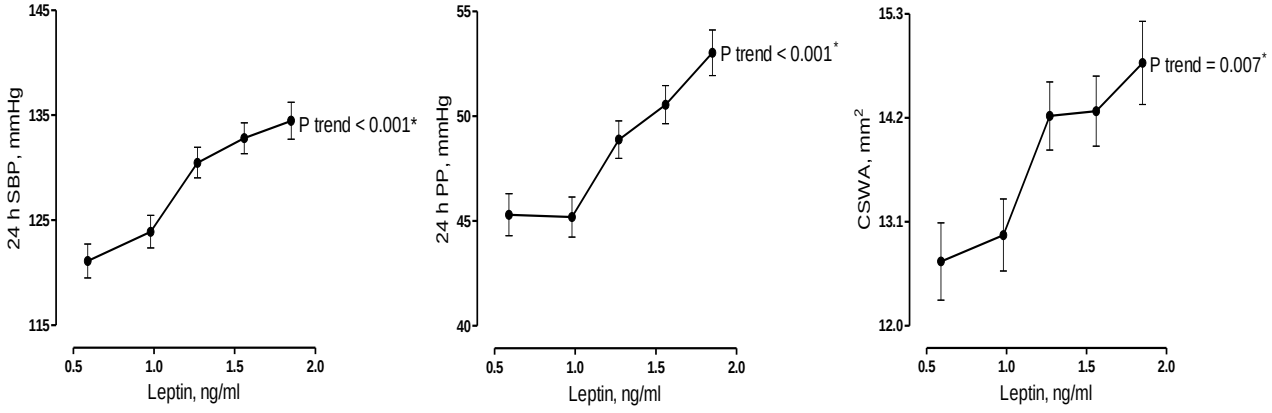

Figure 1 The 24 h SBP, 24 h PP and CSWA by pentiles of leptin levels in the total group adjusted for ethnicity, gender and age. Values are arithmetic mean ± S.E. P denotes significance for trend; *P<0.05 (pentile$_1$ vs pentile$_5$)
Abbreviations: SBP, systolic blood pressure; PP, pulse pressure; CSWA, cross-sectional wall area.

The independent associations between the cardiovascular variables and leptin are shown in Table 4. With adjustments applied for significant covariates, the previously mentioned associations in the total group were confirmed. Positive associations of all cardiovascular variables with leptin were found except with CIMT (P=0.17). When additionally adjusting for body mass index, the positive association of CSWA with leptin remained in the total group (P=0.019), but associations with 24 h SBP, 24 h DBP and 24 h PP disappeared.

Sensitivity analysis

The disappearance of the association between 24 h blood pressure and leptin could possibly be due to the volume loading effect associated with increasing body mass index. To confirm this in single regression analyses, both leptin (r=0.21; P<0.001) and body mass index (r=0.51; P<0.01) correlated with stroke volume, while 24 h SBP (r=0.22; P<0.001) and 24 h DBP (r=0.07; P=0.17) also correlated with stroke volume. The association between 24 h blood pressure and leptin also disappeared after adjusting for stroke volume.

Table 4 Independent associations between cardiovascular variables and leptin.

	24 h SBP, mmHg	24 h DBP, mmHg	24 h PP, mmHg	CIMT (far wall), mm	CSWA, mm²
	Std ß (95 % CI)	Std ß (95 % CI)	Std ß (95 % CI)	Std ß (95 % CI)	Std ß (95 % CI)
Total group (n=407)					
R^2	0.317	0.362	0.106	0.382	0.439
Adjusted R^2	0.305	0.350	0.100	0.370	0.428
Leptin, ng/ml	0.261 (0.146 to 0.377)§	0.144 (0.033 to 0.257)†	0.335 (0.218 to 0.454)§	0.079 (-0.033 to 0.192)	0.121 (0.020 to 0.223)†
Ethnicity (African, Caucasian)	-0.170 (-0.274 to -0.068)‡	-0.203 (-0.303 to -0.104)§	-	-0.139 (-0.234 to -0.042)‡	-
Gender (men, women)	-0.306 (-0.433 to -0.181)§	-0.305 (-0.428 to -0.185)§	-0.223 (-0.346 to 0.101)§	-0.138 (-0.253 to -0.021)†	-0.303 (-0.409 to -0.192)§
Age, years	0.092 (0.0086 to 0.177)†	0.099 (0.018 to 0.180)†	-	0.444 (0.358 to 0.519)§	0.369 (0.286 to 0.444)§
24 h mean arterial pressure, mmHg	-	-	-	0.150 (0.052 to 0.243)‡	0.230 (0.140 to 0.314)§
Total cholesterol, mmol/l	-	-	-	0.091 (0.0034 to 0.175)†	0.060 (-0.018 to 0.136)
Serum glucose, mmol/l	0.208 (0.171 to 0.298)§	0.180 (0.095 to 0.267)§	0.154 (0.057 to 0.252)‡	0.137 (0.049 to 0.222)‡	0.100 (0.017 to 0.181)†
C-reactive protein, mg/l	-	-	-	-	-
Smoking (no, yes)	-0.088 (0.172 to 0.0040)†	-0.090 (-0.171 to -0.0086)†	-	-0.069 (-0.148 to 0.012)*	-0.089 (-0.164 to -0.012)†
Gamma-glutamyl transferase, U/L	0.151 (0.041 to 0.261)‡	0.209 (0.103 to 0.316)§	-	-	-
HIV infection (no, yes)	-	-	-	-	-
Anti-hypertensive medication (no, yes)	-	-	-	-	-0.086 (-0.165 to -0.0061)†
Additionally adjusted for body mass index					
R^2	0.360	0.376	0.168	0.382	0.439
Leptin, ng/ml	-0.037 (-0.197 to 0.123)	-0.025 (-0.183 to 0.132)	-0.043 (-0.164 to 0.077)	0.079 (-0.033 to 0.192)	0.121 (0.020 to 0.223)†

Standardised ß (std ß) reflects the change in the dependent variable for 1 SD change in the independent variable. A larger std ß reflects greater strength of the association.

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; CIMT, carotid intima-media thickness; CSWA, cross-sectional wall area.

*0.1≥P≥ 0.05; †P≤0.05; ‡P≤0.01; §P≤0.001.

Discussion

The main finding of our study is a positive association obtained with leptin and carotid cross-sectional wall area, independent of body mass index. This suggests that higher leptin levels may be an important contributor to the development of atherosclerosis in this group, of which 68% were either overweight or obese.

Previous studies also investigated associations between carotid intima-media thickness and leptin; however contradictory results were found. A correlation between carotid intima-media thickness and leptin was demonstrated in obese individuals,[20] whereas other studies involving elderly overweight Caucasian men[21] and obese children showed no association.[22] In support of our finding, atherosclerosis and thrombus formation was promoted in apolipoprotein E-deficient mice receiving leptin treatment.[23] In the present study, the association of cross-sectional wall area with leptin remained significant even after adjusting for body mass index. This suggests that the increase in cross-sectional wall area was not due to functional changes to normalise tensile stress, which occur with volume loading due to an increase in fat mass,[24] but rather that elevated leptin levels contribute to thickening of the arterial wall via mechanisms that are independent of obesity *per se.* Whatever the mechanism may be, the association discovered between the cross-sectional wall area and leptin remained after adjustment for body mass index.

The association between CSWA and leptin may be explained by multiple mechanisms. *In vitro* studies show that leptin promotes the production of reactive oxygen species either by reducing anti-oxidant enzymes or by increasing fatty acid oxidation.[25,26] In support of these findings, we obtained a strong association between reactive oxygen species and leptin in the present study. Oxidative stress plays an important part during the onset of atherosclerosis.[27] A further atherosclerotic contribution of leptin is to induce smooth muscle cell hypertrophy and proliferation via endothelin-1 and angiotensin II dependent mechanisms.[28] Angiotensin II also increases the production of reactive oxygen species[29] and induces the release of the vasoconstrictor endothelin-1.[30] Leptin also stimulates the secretion of inflammatory markers such as C-reactive protein[31] and interleukin-6[32] that are directly involved with the development of endothelial dysfunction and atherosclerosis.

Although it is unclear which of these mechanisms involving leptin contribute, or contribute the most, to the development of atherosclerosis in our study group, our findings suggest that leptin at least plays an important part. Even though our findings were independent of body mass index and other conventional risk factors, the fact that 68% of the group were either overweight or obese cannot be ignored. Therefore, although leptin seems to contribute directly to atherosclerosis independently of

obesity, it remains the excess adipose tissue that is responsible for the elevated leptin levels and therefore one would expect these effects to be more prominent in overweight and obese individuals.

We also established a positive relationship between ambulatory blood pressure and leptin. This result contributes to controversial findings where associations between blood pressure and leptin were found independent of body mass index in some studies,[33,34] whereas others did not report this.[35] However in the present study, when body mass index was added into our regression model, the associations of 24 h blood pressure and 24 h pulse pressure with leptin disappeared. Possible explanations may be the confounding volume loading effect associated with increasing adiposity which also raises blood pressure.[36,37] This is supported by the correlations of stroke volume with leptin, body mass index and 24 h SBP, possibly explaining the link between blood pressure and leptin.

We also noticed no significant interactions with ethnicity or gender for the associations between the cardiovascular variables and leptin. The lack of interaction may indeed be due to the fact that our study population consisted of school teachers with a similar socio-economic status. The relationship between blood pressure, obesity and socio-economic status is well known[38] and therefore a similar socio-economic status seems to yield similar results, irrespective of ethnicity. In addition, we also investigated ethnic and gender differences in leptin levels. Women had higher leptin levels than men, confirming previous studies.[39,40] On the other hand, previous contradictory results have been found regarding ethnic differences in leptin levels.[41,42] Our results show that Africans have higher leptin levels than Caucasians, but it is noteworthy that the African group also recorded a higher body mass index than their Caucasian counterparts.

Our results support the notion that if the emerging epidemic of obesity in South Africa is left unopposed, the already increasing trends in cardiovascular disease will reach immense proportions. Therefore, future research and clinical studies are needed to fully understand the effects of leptin on the cardiovascular system, and more specifically on the development of hypertension and atherosclerosis. Despite all this uncertainty, leptin treatment to promote weight loss is becoming more popular, which is a major cause for concern.

This study has to be interpreted within the context of its limitations and strengths. It could be questioned whether these groups are comparable due to known ethnic differences in fat distribution.[42] Subcutaneous fat releases more leptin than visceral fat deposits and it is recognized

that Africans possess more subcutaneous fat than their Caucasian counterparts for any given body mass index.[43] In addition, our results were statistically significant, but leptin may contribute to vascular disease risk by interacting with other risk factors. This was a selected target population; therefore results cannot be extrapolated to other population groups. Although our results were consistent after multiple adjustments, we cannot exclude the possibility that our associations were due to residual confounding. This was a cross-sectional study and therefore we cannot infer causality. Apart from this, we conducted a well-designed study under highly controlled conditions.

To conclude, circulating leptin levels was associated with carotid cross-sectional wall area, a marker of atherosclerotic progression, independent of body mass index. The potential implication of this finding is that leptin seems to contribute to the development of atherosclerosis and that care should be taken when administering leptin in the treatment of obesity.

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CHAPTER 4

SUMMARY OF THE MAIN FINDINGS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Introduction

This chapter is a summary of the main findings from the study. The main results will be discussed and compared to relevant literature, after which conclusions and recommendations will be made for future researchers investigating this link between atherosclerosis and leptin.

Summary of the main findings

This study aimed to investigate leptin levels and their associations with cardiovascular function in African and Caucasian men and women. The results indicated that women had higher leptin levels than men, and that Africans had higher leptin levels than Caucasians. Therefore, the first and second hypotheses (as set out on page 14) are accepted. Africans also recorded higher 24 h blood pressure and carotid intima-media thickness than Caucasians; therefore, the third hypothesis is accepted. Lastly, positive associations between 24 h blood pressure and carotid intima-media thickness with leptin were obtained. However, due to lack of interaction of ethnicity and gender with regards to the associations between leptin and the cardiovascular variables, associations were deemed similar for all stratifications and analysed for the total group. Therefore, the last hypothesis is partially accepted.

An additional finding of our study was that the association between carotid artery wall thickness and leptin was independent of body mass index. This result suggests that leptin may contribute in itself to the development of atherosclerosis, perhaps even in lean to overweight individuals who take prescribed leptin products for acute weight loss.

Comparison to relevant literature

The results of the present study confirm and contradict previous studies. Our result regarding gender differences in leptin levels confirms previous findings, where women demonstrate higher leptin levels than men due to higher levels of adiposity.[1,2] Studies also show that Africans have higher leptin levels than Caucasians,[3,4] and these findings were independent of body mass index. However, another study did not find ethnic differences in leptin levels.[2]

There is increasing evidence which suggests that leptin contributes to the pathogenesis of hypertension and atherosclerosis;[5,6] therefore, leptin is also regarded as a possible risk factor for cardiovascular events such as stroke and myocardial infarction.[7,8] In support of this, associations between blood pressure and leptin have been reported,[9] and in some cases, these were independent of body mass index.[10-12] We also obtained associations between 24 h blood pressure and leptin; however, these associations disappeared when adjusting for body mass index.

Contradictory findings regarding the relationship between carotid intima-media thickness and leptin also exist.[13-15] The positive association of cross-sectional wall area with leptin obtained in our study, independent of body mass index, is supported by a previous study which also demonstrated an association with carotid intima-media thickness.[13] However, our result contradicts another previous study involving overweight Caucasian men which did not show associations with leptin and measures of atherosclerosis.[14]

Discussion of main findings

Our main finding, that elevated leptin levels may contribute to the development of atherosclerosis, has worrying implications. It is well known that the African population of South Africa is undergoing urbanisation and subsequently there is an increasing trend towards obesity.[16,17] In support of this, our study group consisted of 68% overweight or obese individuals where Africans demonstrated the highest percentage, namely 36%. It is known that African women record the highest prevalence of obesity in South Africa.[16] This may partly be due to traditional and cultural factors and ways of thinking. Obesity is perceived as a portrayal of wealth, happiness and a negative human immunodeficiency virus status.[16] Keeping this in mind, the stroke mortality rate in Africans is twice as high as that in the Caucasian population,[18] possibly owing to the high obesity rates. Therefore, individuals should be made aware of the potential fatal effects of being overweight or obese and cultural interventions should be considered. In light of the publicity surrounding leptin as a possible weight loss drug, clinical trials should assess the full scope of its cardiovascular effects before leptin supplements/injections can be prescribed to treat obesity.

Chance and confounding

It is important to reflect on factors that might have influenced the results of this study. Although included in the multiple regression models, confounding factors such as ethnicity, gender, age, body mass index, C-reactive protein, smoking, alcohol intake and physical activity could have influenced the results by causing over- or underestimation of the associations between the 24 h blood pressure, cross-sectional wall area and leptin levels investigated in this study. Even though our results were of statistical significance, this does not necessarily indicate physiological significance.

Our study group consisted of African and Caucasian school teachers from the Potchefstroom district in the North West Province of South Africa; therefore, they cannot be regarded as a representation of the rest of the South African population. However, apart from these limitations,

this was a well-designed study conducted under controlled conditions at the metabolic unit, where participants received a standardised meal after which they fasted overnight to ensure fasting leptin levels the next morning.

Conclusion

In conclusion, the carotid cross-sectional wall area was positively associated with circulating leptin levels. These findings were independent of ethnicity, gender, body mass index and significant covariates. The potential implication of this finding is that leptin seems to contribute to the development of atherosclerosis and that care should be taken when considering leptin in the treatment of obesity. Future studies are warranted to confirm these findings.

Recommendations

The following is recommended for future studies:

- Accurate measurement of dietary intake to determine which substances could raise or reduce leptin levels. The effect of sex hormones on leptin synthesis should also be taken into account.
- The relationship between leptin and other adipocytokines such as adiponectin should also be determined.
- Because insulin and glucose metabolism regulate leptin and adiponectin release, insulin sensitivity should be measured.
- Due to the strong association between body mass index and leptin, the study population should be matched for similar body mass index or fat distribution.
- Obesity and the distribution of visceral adipose tissue and subcutaneous adipose tissue should be assessed accurately by use of magnetic resonance imaging or by Dual-Energy X-ray Absorptiometry.
- Associations between markers of sympathetic activity and leptin should also be explored.
- Associations between triglycerides, C-reactive protein, interleukin-6, endothelin-1 and angiotensin II with leptin should be investigated prospectively, to determine more accurately which mechanisms are at work.

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Appendix A

NORTH-WEST UNIVERSITY

POTCHEFSTROOM CAMPUS

SCHOOL FOR PHYSIOLOGY, NUTRITION AND CONSUMER SCIENCES

PARTICIPANT INFORMATION AND CONSENT FORM

PART 1

PRINCIPAL RESEARCHER: Dr Leoné Malan, Subject Group Physiology
PROJECT LEADER: Dr. Leoné Malan, Subject Group Physiology

Associate Researcher(s): The postdoctoral fellow involved in this trial is Dr. Szabolcs Péter. Other persons assisting in the study are Dr. Hugo W. Huisman, Prof. Johannes M. van Rooyen, Prof. Nico T. Malan, Dr R Schutte, Mrs. Carla M.T. Fourie, Mrs. Tina Scholtz (Cardiovascular research group, Physiology), Prof. Salomé Kruger & Dr. Ramoteme Mamabolo, (Physical activity), Proff. Hans de Ridder (Anthropometry), Marié Wissing (Psychology), Linda Brand & Brian Harvey (Pharmacology), Kobus Mentz (Education), Francois van der Westhuizen (Biochemistry), Hester Klopper (Nursing), Nancy Frasure-Smith & Francois Lespérance (Psychology, Canada), Alaa Alkerwi (Epidemiology, Luxembourg), Yackoob Seedat (ECG, Kwazulu Natal), Paul Rheeder (Sonar, Pretoria Univeristy), Drs. Johan Potgieter & Michael Temane & Mr Thumi Khumalo (Psychology), Mrs Gedina de Wet (Nursing).

This Participant Information and Consent Form is **7** pages long. Please make sure you have all the pages.

Your Consent

You are invited to take part voluntarily in this research project.

This participant information document contains detailed information about the research project which has been explained to you verbally. Its purpose is to explain to you as openly and clearly as possible all the procedures involved in this project before you decide whether or not to take part.

Please read this *Participant Information Form* carefully. Feel free to ask questions about any information in the document. You may also wish to discuss the project with a relative or friend or your local health worker. Feel free to do this.

Once you understand what the project is about and if you agree to take part in it, you will be asked to sign the Consent Form. By signing the Consent Form, you indicate that you understand the information and that you give your consent to participate in the research project.

You will be given a copy of the Participant Information and Consent Form to keep as a record.

What is the study about?

The aim of this project is to have an impact on the eventual prevention and treatment of lifestyle diseases in Africans from South Africa. New knowledge regarding the relationship between higher nervous system activity implicating cardiovascular, metabolic and psychological well-being will improve understanding and change strategies at the roots of treatment and prevention of lifestyle diseases.

Our research has shown that lifestyle diseases in urbanised Africans present higher obesity levels, high blood pressure or hypertension prevalence rates and the experiencing of more stress. This pattern is enhanced during psychosocial stress/urbanisation in participants with a specific coping style. Hence the planned SABPA project, which is the first study in South Africa where coping and direct markers of nervous system activity in Africans will be measured.

Purpose of study

The purpose of this study is to investigate biological markers associated with higher sympathetic nervous system activity in urbanised teachers with a specific coping style.

To investigate the relationship between blood pressure, inflammation, obesity, stress and coping in more detail we are going to perform this study in 400 men and women from the North-West province, aged 25-60 years. A comprehensive assessment of the cardiovascular and nervous systems by means of non-invasive painless techniques will be performed and a blood sample will be taken by an experienced research doctor and nurse to determine your blood sugar, cardiovascular, inflammation and stress hormone levels amongst other health markers.

Procedures

All measurements are performed in the Metabolic Unit (lipid clinic) of the University. A researcher has explained the entire procedure in detail and while you are reading this information document you have time to ask questions and to have clarified matters. If you are fine with the explained procedure you are requested to sign a *consent form (at the end of this document). Remember all personal data will be handled with care and remain confidential.

**By consenting to participate in this study, you consent to the storage and later analysis and testing of your stored blood samples for the purposes noted above. Your blood will also be tested for preliminary results on HIV status, since your HIV status may directly influence the main purposes of this study. If you would like to know what your HIV-status is, we will provide it. If tested positive we will refer you to your doctor and he/she will perform the necessary tests which will allow you to apply for chronic medication benefit. Also, the blood cells from your donated blood sample will be used to investigate the molecular genetics of higher nervous system activity and type 2 diabetes in order to enable pre-symptomatic diagnosis of hypertension and diabetes in the long term.*

Why was I chosen? Teachers are exposed to changing curricula and disciplinary problems whilst living in an urbanised environment adding to higher stress experiencing and nervous system activity.

How was I chosen?
Inclusion criteria:
Phase I: 200 black Africans aged 25-60 years (male=100, female = 100)
Phase II: 200 white Africans (n = male, 100 = female) aged 25-60 years.

Exclusion criteria: *pregnancy, lactation, any acute/chronic medication (e.g. high blood pressure, TB/tuberculosis, high sugar/diabetes, arthritis, anti-clotting/stroke factors, epilepsy/mental diseases or being treated for it as well being addicted to the medicine). You can not be included if you have been vaccinated in the previous 3 months and if you are a regular blood donor.*

What will be expected of me?

You, as participant will be screened once by a registered nurse to be eligible complying to the inclusion criteria. The following procedures will be followed:

- Recruitment, screeening and informed sessions with all participants will be done two months prior to the study (October - November 2007, Phase I, and November, 2008, Phase II) and informed consent forms will be signed.
- After selection of all participants, the details of the project will be discussed with you in English or your home language, i.e. what the exact objectives of the study are, what procedures will be taken and what will be expected from each of you (e.g. overnight stay, resting blood pressure procedures and fasting urine and blood samples are required, importance of complying with the correct sampling methods, incentives). You will be given the opportunity to ask questions.
- Data collection for each participant will involve two days (15min in the morning and 2½ hours in the evening) on Day I; and 2 hours on Day II):

DAY I

- On day I at 07:00, the blood pressure apparatus, which will measure your blood pressure and heart function as well as a physical activity meter will be applied to your arm and waist at your school and you can then resume your normal daily activities. In the afternoon you must complete the Neethling Brain Instrument questionnaire which measures thought processes of the brain.
- At the end of Day I (± 16:30) you will be transported from your schools to overnight in the Metabolic Unit Research Facility of the North-West University. This unit is a research unit for human studies and equipped with 10 well furnished bedrooms, a kitchen, two bathrooms and a television room. Each of you will be subjected to the following procedures:
  - At the end of Day I between ± 17:15 and 18:00 you will be welcomed and each of you will receive your own private bedroom.
  - The procedures, which will be done, will be explained again and each of you will then complete a general socio-demographic health questionnaire. Afterwards you will receive dinner.
  - After dinner, psychological questionnaires will be completed under supervision of registered education specialists and psychologists. Completion of questionnaires will take approximately 40 min, including a break of 20 minutes with coffee/tea and biscuits. This willl be you last meal for Day I as you must be fasting on Day II for obtaining good results.
  - Thereafter, you can relax and watch television or socialise with your c-participants. It will be wise to go to bed not later than 22:00 as the blood pressure apparatus will take measurements every hour during the night and it can be tiring.

DAY II

- At 06:45 on Day II the AMBP will be removed and an urine sample collected. Once this has been done you will be directed to the anthropometric station where your weight, height and body circumferences will be measured.
- The next station involves the blood pressure measurement station. Whilst in a sitting position your blood pressure will be taken in duplicate with the sphygmomanometer (the same as used at clinics) with a resting period of 5 minutes in between. Our registered research doctor/nurse will take a fasting saliva sample as well as a blood sample of 45ml from a vein in your dominant arm. The infusion set will be left in your arm to lessen the effect of inserting a needle again for blood sampling after exposure to the two stressors. A small amount of diluted heparin will be left in the infusion set in your arm to prevent clotting.

Next the cardiovascular measurements will follow consisting of three separate procedures:

- The 1st measurement involves an ECG apparatus, which measures heart function, with 12 leads, which will be placed into position on your rib cage/front part of the body.
- The 2nd measurements are non-invasive and will be done by means of the Finometer device which also involves the assessment of heart functioning such as pulse (beats per minute), stroke volume (blood volume ejected by the heart per beat), cardiac output (blood volume ejected by the heart per minute), total peripheral resistance (resistance against the blood flow created by small arteries), central resistance (resistance against which the heart has to work while ejecting the blood into the aorta) as well as the elasticity of your large arteries (compliance). For this procedure a blood pressure cuff will be placed around your left arm and middle finger which will be inflated and stepwise deflated. You will not have more discomfort than during a common blood pressure measurement. This will take about 5 minutes.
- The stressor application procedure follows: You will now be exposed to a stressor for 1 minute whilst your blood pressure and ECG will still be taken. After exposure a saliva and blood sample (45ml) will be taken. After 10 minutes another saliva sample will be taken. Then the stressor application procedure will be repeated with the second stressor.
- At another station your 3rd measurement includes the assessment of pulse wave velocity, i.e. how fast your blood travels through your arteries. This measurement gives us an indication about how stiff your vessel walls are. The stiffer your vessel wall is the faster the blood travels from one point of your body to another. These painless measurements will require two technicians using blunt probes (tonometer) putting light pressure on the neck and on the foot to measure the velocity of the pulse waves. This takes only a few minutes. An ultrasound device will be taken of your arteries in the neck with a blunt probe to indicate the intrinsic thickness of your arteries which contributres to high blood pressure.

  The two stressors you will be exposed to for one minute include:

  1. The *Colour-Word-Conflict Chart (applied for 1 minute)* is written in various colours. You must say or select the ink colour rather than the name of the colour spelled out by the word. A sliding scale with monetary incentives (maximum of R55.00) will be given if you can complete reading the chart.
  2. *The Cold Pressor Test (Foot) (applied for 1 minute)*: Immersion of your foot up to the wrist in ice water (4 degrees Celcius). As the cold can make you hold your breath you must quietly count to yourself during cold exposure to breath more rythmic.

- You have reached the end of the sampling phase.
- **Thank you for your participation! You now will have the opportunity to shower and a take away breakfast will be given**.
- Immediate feedback on your HIV/AIDS status, obesity, blood pressure and blood glucose/sugar values will be given. *HIV/AIDS post-test counselling will be arranged if you are tested positive.*
- You are now transported back to your school and after one week you will receive your Neethling Brain Instrument and 24-hour blood pressure reports.

Possible Risks

The measurements performed in our study will include only non-invasive techniques that are not expected to reveal any risks but might cause little discomfort. The taking of blood samples is an invasive procedure with a minimal risk of bleeding. Thus the procedure may cause only a few

seconds of light discomfort. All tests will be performed by experienced research nurses of our department. There may be additional unforeseen or unknown risks.

Precautions to protect the participant

The Metabolic Unit facility of the NWU is fully equipped, and in case of an emergency which could not be handled by the registered nurse, the supervising medical doctor Emile Kotzé will be contacted. Dr. Kotzé was notified before the study commenced that this study will be taking place, and that there is a slight possibility that he may be contacted. Supporting medical treatment care facilities will be at hand anytime if needed.

Other Treatments Whilst on Study

It is important to tell the research staff about any treatments or medications you may be taking, including non-prescription medications, vitamins or herbal remedies during your participation in the study.

Incentives

1. All teachers will receive feedback on their health profile and if necessary references will be given to physicians/clinics/hospitals.
2. Printout feedback on 24 hour blood pressure monitoring report (normally costing R637.60), sonar of the artery (R1200.00), resting ECG (R600.00) and other variables (R500.00). Your benefit of participation is a comprehensive assessment of the cardiovascular and metabolic condition including investigation of blood pressure, inflammatory status and psychological well-being. These examinations will help us to assess the degree of vascular impairment of the arteries and to predict your risk of possible cardiovascular events such as heart attacks and stroke. The results may assist your doctor in decision making for further treatment or for instituting preventive measures. Our study will also contribute to the identification of possible factors leading to high blood pressure. As 24 hour ambulatory blood pressure monitoring is required for the diagnosis of hypertension, medical aids insist on this method of diagnosis to qualify for chronic medication. Additional testing could also reveal illnesses of a chronic nature and would serve as a motivation to qualify for chronic medication, such as metabolic syndrome, anti-inflammatory and cholesterol-lowering drugs.
3. Monetary incentive on completion of the colour word conflict chart (± R55.00).
4. Dinner and breakfast (± R24.00).
5. Neethling Brain Instrument profiles done by registered user of the Whole Brain (normally costing ± R350.00).
6. Coping skills workshop will be arranged on request.

Privacy, Confidentiality and Disclosure of Information

By consenting to participate in this study, you consent to the storage and later analysis and testing of your stored blood samples for purposes noted above. Your blood samples will be discarded immediately after analysis. All information provided by you and the results of tests will be treated in the strictest confidence, and will only be used for the purpose of this research project. It will only be disclosed with your permission, except as required by law. The results of your medical tests will be labelled only with a code number, and will be stored separately from any identifying information. When the results are analysed we will be looking for differences between groups of people, not at the results of individuals. No information that could identify any person taking part in the study will be revealed when the results are reported.

Participation is Voluntary

Participation in any research project is voluntary. If you do not wish to take part you are not obliged to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with the North-West University.

Before you make your decision, a member of the research team will be available so that you can ask any questions you have about the research project. You can ask for any information you want. Sign the Consent Form only after you have had a chance to ask your questions and have received satisfactory answers.

If you decide to withdraw from this project, please notify a member of the research team before you withdraw.

Ethical Guidelines

This project will be carried out according to Ethical Guidelines of the Helsinki declaration from 2000, with additional notes in 2002. This statement has been developed to protect the interests of people who agree to participate in human research studies.

The ethical aspects of this research project have been approved by the Human Research Ethics Committee of ***North-West University Potchefstroom.***

Further Information or Any Problems

If you require further information or if you have any problems concerning this project, you can contact the principal researcher or *the other* researchers responsible for this project.

Dr Leoné Malan (018-299 2438) Signature:

Sr. Chrissie Lessing (018-299 2480) Project Leader: Dr Leoné Malan

PART 2

To the subject signing the consent as in part 3 of this document

You are invited to participate in a research project as described in paragraph 2 of Part 1 of this document. It is important that you read/listen to and understand the following general principles, which apply to all participants in our research project:Participation in this project is voluntary.

1. **It is possible that you personally will not derive any benefit from participation in this project, although the knowledge obtained from the results may be beneficial to other people.**

2. **You will be free to withdraw from the project at any stage without having to explain the reasons for your withdrawal. However, we would like to request that you would rather not withdraw without a thorough consideration of your decision, since it may have an effect on the statistical reliability of the results of the project.**

3. **The nature of the project, possible risk factors, factors which may cause discomfort, the expected benefits to the subjects and the known and the most probable permanent consequences which may follow from your participation in this project, are discussed in Part 1 of this document.**

4. **We encourage you to ask questions at any stage about the project and procedures to the project leader or the personnel, who will readily give more information. They will discuss all procedures with you.**

5. **We require that you indemnify the University from any liability due to detrimental effects of treatment by University staff or students or other subjects to yourself or anybody else. We also require indemnity from liability of the University regarding any treatment to yourself or another person due to participation in this project, as explained in Part 1. Lastly it is required to abandon any claim against the University regarding treatment of yourself or another person due to participation in this project as described in Part 1.**

6. **If you are married, it is required that your spouse abandon any claims that he/she could have against the University regarding treatment or death of yourself due to the project explained in Part 1.**

PART 3

Consent

Title of the project:
"THE SABPA STUDY (**S**YMPATHETIC ACTIVITY AND **A**MBULATORY **B**LOOD **P**RESSURE IN **A**FRICANS)".

I, the undersigned …… (full names) read/listened to the information on the project in PART 1 and PART 2 of this document and I declare that I understand the information. I had the opportunity to discuss aspects of the project with the project leader and I declare that I participate in the project as a volunteer. I hereby give my consent to be a subject in this project.

(Signature of the subject)

Signed at ..... on ……2008

Witnesses

1. .....

2. .....

Signed at ..... on ……2008

Appendix B

SABPA Project

General Health and Sociodemographic Questionnaire

2008

PARTICIPANT NUMBER ☐☐☐☐☐☐☐☐

Gender ☐

RACE White ☐ Black ☐ Indian ☐ Coloured ☐

Date of BIRTH ☐☐ ☐☐ ☐☐☐☐

HOUSE N: P.BOX N: .....

STREET: .....

Post Code: .....TOWN.....

MOBILE phone number.....

P_DUR ☐☐ ☐ Number of years staying in Potchefstroom.

Question 1:

Marital status.

MS_SI ☐ Unmarried

MS_SIP ☐ Unmarried, living with partner

MS_MA☐ Married, living with "legal" wife/husband

MS_MAP ☐ Married, partner other than "legal" husband/wife

MS_DI ☐ Divorced, not living with new partner

MS_DIP ☐ Divorced, living with new partner

MS_WW ☐ Widow or widower, not living with new partner

MS_WWP ☐ Widow or widower, living with new partner

Question 2: Education

Still attending school?

SC_NOW ☐ Now ?

SC_LOC ☐☐☐☐ ☐ School or institution

EDU DI ☐ Completed DIPLOMA

EDU DE ☐ Completed DEGREE

Question 3: Past occupation.

P_HINS ☐ Long-lasting health problems

P_DUR ☐☐ ☐ Number of years

P_P_LOC ☐☐☐☐☐ ☐ Address

Question 4:

SALARY ☐ Employee receiving salary

S_FULL ☐ Full-time basis

S_PART ☐ Part-time basis

S_SUBE ☐☐☐ Persons subordinated to you

---**Question 5:**

EDU DI ☐ DIPLOMA

EDU DE ☐ DEGREE

EDU WW ☐ Hours of work per week

Question 6: (Family members alive)

FH_F ☐ Father

FH_GFf☐ Grandfather (father's side)

FH_GMf ☐ Grandmother (father's side)

FH_M ☐ Mother

FH_GFm ☐ Grandfather (mother's side)

FH_GMm ☐ Grandmother (mother's side)

FH_Ch ☐ Children

FH_GCh ☐ Grandchildren

FH_BSf☐ Brothers or sisters of your father

FH_BSm ☐ Brothers or sisters of your mother

FH_BS ☐ Own brothers or sisters

Question 7:

C_DIS ☐ Disease affecting your heart or blood vessels

C_COD1 Disease ☐☐ ☐☐☐☐ C_BMY1 Starting date ☐☐☐☐

C_EMY1 Date of cure ☐☐☐☐ C_NAGP1 Treating physician .....*…….

C_COD2 Disease ☐☐ ☐☐☐☐ C_BMY2 Starting date ☐☐☐☐

C_EMY2 Date of cure ☐☐☐☐ C_NAGP2 Treating physician .....*…….

C_COD3 Disease ☐☐ ☐☐☐☐ C_BMY3 Starting date ☐☐☐☐

C_EMY3 Date of cure ☐☐☐☐ C_NAGP3 Treating physician .....*…….

C_COD4 Disease ☐☐ ☐☐☐☐ C_BMY4 Starting date ☐☐☐☐

C_EMY4 Date of cure ☐☐☐☐ C_NAGP4 Treating physician .....*…….

Question 8:

K_DIS ☐ Disease affecting your kidneys or urinary tract

K_COD1 Disease ☐☐ ☐☐☐☐ K_BMY1 Starting date ☐☐☐☐

K_EMY1 Date of cure ☐☐☐☐ K_NAGP1 Treating physician .....*……

K_COD2 Disease ☐☐ ☐☐☐☐ K_BMY2 Starting date ☐☐☐☐

K_EMY2 Date of cure ☐☐☐☐ K_NAGP2 Treating physician .....*…….

	Disease		Starting date
K_COD3	☐☐ ☐☐☐☐	K_BMY3 ☐☐☐☐	

	Date of cure		Treating physician
K_EMY3	☐☐☐☐	K_NAGP3.....…*……..	

	Disease		Starting date
K_COD4	☐☐ ☐☐☐☐	K_BMY4 ☐☐☐☐	

	Date of cure		Treating physician
K_EMY4	☐☐☐☐	K_NAGP4 .....…*……..	

Question 9:

L_DIS ☐ Kidney stones or stones in you urinary tract

L_REPC ☐ Repeated pain attacks

L_EVAC ☐ Passed a stone with urine

L_OPER ☐ Surgical treatment

L_NOW☐ Still suffering from kidney stones or stones in urinary tract

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Question 10:

DIABET ☐ Diabetes

D_DIET☐ Diet and avoiding sweet foodstuffs

D_ORAL ☐ Pills

D_INS ☐ Insulin

Question 11:

HYPERT ☐ Hypertension

HY_MY ☐☐☐☐ When ?

HY_Th ☐ Treatment

Question 12:

DISEAS ☐ Currently in good health

Disease	Starting date

DS_CD1 ☐☐ ☐☐☐☐ DS_BMY1 ☐☐☐☐

Date of cure Treating physician
DS_EMY1 ☐☐☐☐ DS_NAGP1.....……*……..

Disease Starting date
DS_CD2 ☐☐ ☐☐☐☐ DS_BMY2 ☐☐☐☐

Date of cure Treating physician
DS_EMY2 ☐☐☐☐ DS_NAGP2 .....……*…….

Disease Starting date
DS_CD3 ☐☐ ☐☐☐☐ DS_BMY3 ☐☐☐☐

Date of cure Treating physician
DS_EMY3 ☐☐☐☐ DS_NAGP3 .....……*……..

Disease Starting date
DS_CD4 ☐☐ ☐☐☐☐ DS_BMY4 ☐☐☐☐

Date of cure Treating physician
DS_EMY4 ☐☐☐☐ DS_NAGP4 .....……*…….

Disease Starting date
DS_CD5 ☐☐ ☐☐☐☐ DS_BMY5 ☐☐☐☐

Date of cure Treating physician
DS_EMY5 ☐☐☐☐ DS_NAGP5.....……*…….

Disease Starting date
DS_CD6 ☐☐ ☐☐☐☐ DS_BMY6 ☐☐☐☐

Date of cure Treating physician
DS_EMY6 ☐☐☐☐ DS_NAGP6.....……*……..

Question 13:

D_HYPT ☐ Drugs to lower blood pressure

DH_NOW ☐ Now ?

Name of drug Tablets/day/dosage
DH_CD1 ☐☐ ☐☐☐☐ DH_DO1 ☐☐

Name of drug Tablets/day
DH_CD2 ☐☐ ☐☐☐☐ DH_DO2 ☐☐

	Name of drug		Tablets/day/dosage
DH_CD3	☐☐ ☐☐☐☐	DH_DO3 ☐☐	
DH_CD4	☐☐ ☐☐☐☐	DH_DO4 ☐☐	

Question 14:

D_DIUR ☐ Diuretics

DD_NOW ☐ Now ?

	Name of drug		Tabets/day/dosage
DD_CD1	☐☐ ☐☐☐☐	DD_DO1 ☐☐	
DD_CD2	☐☐ ☐☐☐☐	DD_DO2 ☐☐	
DD_CD3	☐☐ ☐☐☐☐	DD_DO3 ☐☐	
DD_CD4	☐☐ ☐☐☐☐	DD_DO4 ☐☐	

Question 15:

D_ANAL ☐ Taking pain-killers

DA_YE ☐☐ How many years ?

DA_SAL ☐ Salicylic acid (Disprin)

DA_PAR ☐ Paracetemol (Grand-Pa)

DA_OTH ☐ Analgesic drugs for arthritis (Brufen)

	Name of drug		Units/week
DA_CD1	☐☐ ☐☐☐☐	DA_DO1 ☐☐	
DA_CD2	☐☐ ☐☐☐☐	DA_DO2 ☐☐	
DA_CD3	☐☐ ☐☐☐☐	DA_DO3 ☐☐	
DA_CD4	☐☐ ☐☐☐☐	DA_DO4 ☐☐	

Question 16:

DR_2WK ☐ Medication during last 2 weeks

	Name of medicine		Units/day
DR_CD1	☐☐ ☐☐ ☐☐☐☐	DR_DO1 ☐☐	
DR_CD2	☐☐ ☐☐ ☐☐☐☐	DR_DO2 ☐☐	
DR_CD3	☐☐ ☐☐ ☐☐☐☐	DR_DO3 ☐☐	
DR_CD4	☐☐ ☐☐ ☐☐☐☐	DR_DO4 ☐☐	
DR_CD5	☐☐ ☐☐ ☐☐☐☐	DR_DO5 ☐☐	
DR_CD6	☐☐ ☐☐ ☐☐☐☐	DR_DO6 ☐☐	

Question 17:

T_NOW ☐ Currently smoking

T_CTf ☐☐ Cigarettes with filter per day

T_CT ☐☐ Cigarettes without filter per day

T_CTgt ☐☐☐ Grams of tobacco per day

T_PIgt ☐☐☐ Grams of tobacco per week for pipe

T_SCgr ☐☐ Small cigars per week

T_Cgar ☐☐ Cigars per week

T_AGE ☐☐ When started smoking ? (age)

T_INHA ☐ Inhalation

Question 18:

T_P_PAST ☐ Smoking in the past

T_P_1CDY ☐ At least one cigarette per day during one year

T_P_AGE ☐☐ Age at which participant quitted smoking

T_P_CTf ☐☐ Cigarettes with filter per day

T_P_CT ☐☐ Cigarettes without filter per day

T_P_CTgt ☐☐☐ Grams of tobacco per day

T_P_PIgt ☐☐☐ Grams of tobacco per week for pipe

T_P_SCgr ☐☐ Small cigars per week

T_P_Cgar ☐☐ Cigars per week

T_P_WHY ☐☐ Reason to stop smoking

Question 19:

E_NOW ☐ Current consumption alcoholic beverages

E_BEER ☐☐ Glasses of beer per day

E_TBEER ☐☐ Glasses of traditional beer per day

E_WINE ☐☐ Bottles of wine per week

E_THLOKd ☐☐ Boxes of Thlokwe per day

E_THLOKw ☐☐ Boxes of Thlokwe per week

E_SPIRITt ☐☐ Tot Spirits per day

E_SPIRITb ☐☐ Bottle of Spirits per week

E_LIQR☐☐ Bottle of Liquor per week

E_AGE ☐☐ When started drinking alcohol regularly ? (age)

Question 20:

E_P_PAST ☐ Consumption of alcoholic beverages in the past

E_P_AGE ☐☐ When stopped ? (age)

E_BEER ☐☐ Glasses of beer per day

E_TBEER ☐☐ Glasses of traditional beer per day

E_WINE ☐☐ Bottles of wine per week

E_THLOKd ☐☐ Boxes of Thlokwe per day

E_THLOKw ☐☐ Boxes of Thlokwe per week

E_SPIRITt ☐☐ Tot Spirits per day

E_SPIRITb ☐☐ Bottle of Spirits per week

E_LIQR☐☐ Bottle of Liquor per week

E_P_WHY ☐☐ Why stopped consuming alcoholic beverages ?

Question 21:

C_NOW ☐ Now consumption of caffeine-containing beverages

C_REG ☐☐ Cups of coffee

C_COKE ☐☐ Glasses of Coca-cola

C_OTH ☐☐ Other

C_TEA ☐ Tea

C_DECAF ☐ Decaffeinated coffee

C_DECA_N ☐☐ Number of cups of decaffeinated coffee per day

Question 22:

M-NOW ☐ Periods

Question 23:

DCCET ☐ Ever taken "the pill" ?

DC_NOW ☐ “The pill” now ?

DC_COD ☐☐☐☐☐☐ Name of "the pill"

DC_YE ☐☐☐☐ How long ? (years, months)

Question 24:

PR_PST ☐ Pregnant before

PR_N ☐☐ Number of pregnancies

PR_ABO ☐ Number of miscarriages

PR_LIB ☐ Children born alive

PR_STB ☐ Children stillborn

Question 25:

M_NOW ☐ Still periods

M_IRYE ☐☐☐☐ Since when irregular periods ?

M_DISYE ☐☐☐☐ Since when periods completely disappaeared ?

M_P_SPON ☐ Spontaneous disappearance

M_P_HYST ☐ Removal of only womb

M_HYSTYE ☐☐☐☐ Date (month/year)

M_P_OVRR ☐ Removal of only right ovary

M_OVRRYE ☐☐☐☐ Date (month/year)

M_P_OVRL ☐ Removal of only left ovary

M_P_OVR2 ☐ Removal of both ovaries

M_OVR2YE ☐☐☐☐ Date (month/year)

M_P_ORHR ☐ Removal of right ovary together with womb

M_ORHRYE ☐☐☐☐ Date (month/year)

M_P_OLHR ☐ Removal of left ovary together with womb

M_OLHRYE ☐☐☐☐ Date (month/year)

M_P_HRT ☐ Removal of both ovaries and womb

M_HRTstart ☐☐☐☐ Date (month/year)

MS_COD1 ☐☐☐☐ Underlying disease 1

MS_COD2 ☐☐☐☐ Underlying disease 2

MS_COD3 ☐☐☐☐ Underlying disease 3

M_P_DRUG ☐ Periods suppressed by taking "the pill"

MD_P_COD ☐☐ ☐☐☐☐ Name of "the pill"

MD_P_MN ☐☐☐ Number of months

Question 26:

E_EXCS ☐ Results sent only to yourself

R-EXGP ☐ Results sent only to your family doctor

E_S_GP ☐ Results sent to yourself and your family doctor

Question 27:

C-GP ☐ Consent to contact the subject's physician(s)

Appendix C

Subject Group Physiology

AMBP card 2008

SURNAME----------

NAME----------

CONTACT NAME AND NUMBER----------

DBIRTH Day☐☐ Month☐☐ Year☐☐☐☐ date of birth

CPNBR☐☐☐☐☐☐☐☐☐☐ identification number

DATABP_S ☐☐ ☐☐ ☐☐☐☐ date of ABP recording

Please indicate if you experience/do any of the following during the 24 hour recording:

C_DIZ ☐ **dizziness** **1 = yes, 2 = no**

TDIZ_S1 ☐☐☐☐ time of start of dizziness 1

TDIZ_E1 ☐☐☐☐ time of end of dizziness 1

TDIZ_S2 ☐☐☐☐ time of start of dizziness 2

TDIZ-E2 ☐☐☐☐ time of end of dizziness 2

C_FAT ☐ fatigue 1 = yes, 2 = no

TFAT_S1 ☐☐☐☐ time of start of fatigue 1

TFAT_E1 ☐☐☐☐ time of end of fatigue 1

TFAT_S2 ☐☐☐☐ time of start of fatigue 2

TFAT_E2 ☐☐☐☐ time of end of fatigue 2

C_VIS ☐ **visual problems** **1 = yes, 2 = no**

TVIS_S1 ☐☐☐☐ time of start of visual problems 1

TVIS_E1 ☐☐☐☐ time of end of visual problems 1

TVIS_S2 ☐☐☐☐ time of start of visual problems 2

TVIS_E2 ☐☐☐☐ time of end of visual problems 2

C_HEAD ☐ **headache** **1 = yes, 2 = no**

THEAD_S1 ☐☐☐☐ time of start of headache 1

THEAD_E1 ☐☐☐☐ time of end of headache 1

THEAD_S2 ☐☐☐☐ time of start of headache 2

THEAD_E2 ☐☐☐☐ time of end of headache 2

C_HOT ☐ hot flushes 1 = yes, 2 = no

THOT_S1 ☐☐☐☐ time of start of flushes 1

THOT_E1 ☐☐☐☐ time of end of flushes 1

THOT_S2 ☐☐☐☐ time of start of flushes 2

THOT_E2 ☐☐☐☐ time of end of flushes 2

C_VOM☐ nausea and/or vomiting 1 = yes, 2 = no

TVOM_S1 ☐☐☐☐ time of start of nausea 1

TVOM_E1 ☐☐☐☐ time of end of nausea 1

TVOM_S2 ☐☐☐☐ time of start of nausea 2

TVOM_E2 ☐☐☐☐ time of end of nausea 2

C_PALP ☐ palpitations/fast heart beat 1 = yes, 2 = no

TPALP_S1 ☐☐☐☐ time of start of palpitations 1

TPALP_E1 ☐☐☐☐ time of end of palpitations 1

TPALP_S2 ☐☐☐☐ time of start of palpitations 2

TPALP_E2 ☐☐☐☐ time of end of palpitations 2

C_SYNC ☐ syncope/faint feeling 1 = yes, 2 = no

TSYNC_S1 ☐☐☐☐ time of start of syncope 1

TSYNC_E1 ☐☐☐☐ time of end of syncope 1

TSYNC_S2 ☐☐☐☐ time of start of syncope 2

TSYNC_E2 ☐☐☐☐ time of end of syncope 2

C_OTHICD ☐☐☐☐☐ ICD code for other symptoms if present

TOTH_S1 ☐☐☐☐ time of start of other 1

TOTH_E1 ☐☐☐☐ time of end of other 1

TOTH_S2 ☐☐☐☐ time of start of other 2

TOTH_E2 ☐☐☐☐ time of end of other 2

A_LPH ☐ light physical activity 1 = yes, 2 = no

TLPH_S1 ☐☐☐☐ time of start of LPH 1

TLPH_E1 ☐☐☐☐ time of end of LPH 1

TLPH_S2 ☐☐☐☐ time of start of LPH 2

TLPH_E2 ☐☐☐☐ time of end of LPH 2

TLPH_S3 ☐☐☐☐ time of start of LPH 3

TLPH_E3 ☐☐☐☐ time of end of LPH 3

A_PH ☐ **physical effort** **1 = yes, 2 : no**

TPH_S1 ☐☐☐☐ time of start of physical effort 1

TPH_E1 ☐☐☐☐ time of end of physical effort 1

TPH_S2 ☐☐☐☐ time of start of physical effort 2

TPH_E2 ☐☐☐☐ time of end of physical effort 2

TPH_S3 ☐☐☐☐ time of start of physical effort 3

TPH_E3 ☐☐☐☐ time of end of physical effort 3

S-SST ☐ **slightly stressed** **1 = yes, 2 = no**

TSST_S1 ☐☐☐☐ time of start of slightly stressed 1

TSST-E1 ☐☐☐☐ time of end of slightly stressed 1

TSST_S2 ☐☐☐☐ time of start of slightly stressed 2

TSST-E2 ☐☐☐☐ time of end of slightly stressed 2

TSST_S3 ☐☐☐☐ time of start of slightly stressed 3

TSST-E3 ☐☐☐☐ time of end of slightly stressed 3

S_ST ☐ **stress** **1 = yes, 2 = no**

TST_S1 ☐☐☐☐ time of start of stress 1

TST_E1 ☐☐☐☐ time of end of stress 1

TST_S2 ☐☐☐☐ time of start of stress 2

TST_E2 ☐☐☐☐ time of end of stress 2

TST_S3 ☐☐☐☐ time of start of stress 3

TST_E3 ☐☐☐☐ time of end of stress 3

TSLEEP ☐☐☐☐ **time of sleep**

TGUP ☐☐☐☐ **time of getting-up**

THSLEEP ☐☐☐☐ **hours of sleep per night**

TASLEEP ☐☐☐☐ **hours awake/can't sleep per night**

TMEAL ☐☐☐☐ **time of main meal**

DR_CD1 ☐☐☐☐☐☐ drug 1 (coded as in the standard questionnaire)

DR_DO1 ☐☐ amount of tablets taken

TIMDR1 ☐☐☐☐ time at which the medication was taken

DR_CD2 ☐☐☐☐☐☐ drug 2 (coded as in the standard questionnaire)

DR_DO2 ☐☐ amount of tablets taken

TIMDR2 ☐☐☐☐ time at which the medication was taken

DR_CD3 ☐☐☐☐☐☐ drug 3 (coded as in the standard questionnaire)

DR_DO3 ☐☐ amount of tablets taken

TIMDR3 ☐☐☐☐ time at which the medication was taken

DR_CD4 ☐☐☐☐☐☐ drug 4 (coded as in the standard questionnaire)

DR_DO4 ☐☐ amount of tablets taken

TIMDR4 ☐☐☐☐ time at which the medication was taken

DR_CD5 ☐☐☐☐☐☐ drug 5 (coded as in the standard questionnaire)

DR_DO5 ☐☐ amount of tablets taken

TIMDR5 ☐☐☐☐ time at which the medication was taken

DR_CD6 ☐☐☐☐☐☐ drug 6 (coded as in the standard questionnaire)

DR_DO6 ☐☐ amount of tablets taken

TIMDR6 ☐☐☐☐ time at which the medication was taken.

Appendix D

INFORMED CONSENT FOR HIV RAPID TESTING

I, hereby give permission that my blood may be taken for HIV testing.

The following has been explained to me:

1. The need for testing;
2. What HIV/AIDS is;
3. The advantages and disadvantages of testing;
4. How the rapid test will be done;
5. The result of this test will not appear on my medical records;
6. Who will inform me of the results;
7. What will happen should I test positive;
8. That a repeat test may be necessary;

Signed (Client)…… Date:

Signed (Witness)…… Date:

Post-test counselling – a negative result

1. Check that you have the correct result.
2. Assess whether or not the client is ready to get his or her result.
3. Give the result calmly and professionally.
4. Wait for the client's response and normalise it.
5. Check that the client understands the test result and the meaning of the window period.
6. Counsel the client for risk reduction. (This is crucial.)
7. Encourage the client to ask any questions.

Post-test counselling – giving a positive result

First session

1. Check that you have the correct result.
2. Assess whether or not the client is ready to get his or her result.
3. Give the result calmly and professionally.
4. Wait for the client's response and normalise it. A wide range of responses are normal and can include shock, disbelief, anger, fear, anxiety, agitation, blame or even seeming indifference.
5. Check that the client understands the test result.
6. Be there emotionally for the client and contain his or her emotions. (This is crucial.) Help work through the feelings.
7. Understand the client faces multiple losses.
8. Counsel for risk reduction.
9. Plan how client will cope with next 24 hours.
10. Give appropriate support contact numbers.
11. Assess suicide risk.
12. Allow client to ask questions.
13. Make follow-up appointment.

Second session

1. Allow the client to lead the session.
2. Encourage the client to work through his or her response to the positive diagnosis.
3. Explore relationship issues and possibility of disclosure.
4. Discuss welfare options.
5. Explore concerns about current children and about having children.
6. Explore treatment options.
7. Explore lifestyle changes – counsel for positive lifestyle.
8. Work to empower and build hope.
9. Make client aware of resources.
10. Assess suicide risk.
11. Allow client to ask questions.