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The Relationship between Cortisol, C-Reactive Protein and Hypertension in the Development of Cardiovascular Dysfunction in African and Caucasian women: the POWIRS Study.

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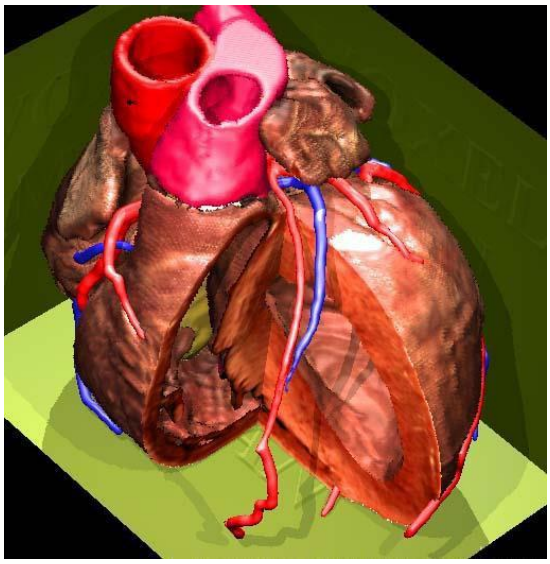

Dissertation submitted in fulfilment of the requirements for the degree Magister Scientiae in Physiology at the Potchefstroom Campus of the North-West University

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Co-supervisor: **Prof JM van Rooyen**

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DECLARATION BY AUTHORS

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

NAME	ROLE IN THIS STUDY
Ms C Tolmay (Physiologist)	Responsible for literature searches, statistical analyses, processing of data, design and planning of manuscript, interpretation of results and writing of manuscript.
Dr L Malan (Physiologist)	Supervisor. Supervised the writing of the manuscript, initial planning and design of manuscript, technical advice regarding literature, statistical analyses and interpretation of results.
Dr JM van Rooyen (Physiologist)	Co-supervisor. Supervised the writing of the manuscript, technical advice regarding the literature, statistical analyses, and interpretation of results.

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

I declare that I have approved the above-mentioned manuscript, that my role in the study, as indicated above, is representative of my actual contribution and that I hereby give consent that it may be published as part of the MSc dissertation of Ms C Tolmay.

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Dr L Malan Dr JM van Rooyen

AFRIKAANSE TITEL: Die verhouding tussen kortisol, C-reaktiewe proteïen en hipertensie in die ontwikkeling van kardiovaskulêre disfunksie in Afrika- en Kaukasiër vrouens: die POWIRS-studie.

OPSOMMING

otivering: C-reaktiewe proteïen (hs-CRP) en ander risikofaktore soos kortisol en obesiteit in die diagnose van kardiovaskulêre disfunksie (KVD) in Afrika- en Kaukasiër vrouens word toenemend belangrik as die toename in hipertensie in hierdie groepe in aanmerking geneem word. Onlangse studies het sommige aspekte van hierdie risikofaktore asook die rol wat hul speel in hipertensie en moontlike toekomstige risiko vir kardiovaskulêre siekte, ondersoek.

CHs-CRP is geassosieer met die verhoogde voorkoms van hipertensie en obesiteit. Kortisol *per se* is ook verwant aan die ontwikkeling van beide hipertensie en die hipotalamus-pituïtêre-adrenaalkorteks respons. Desnieteenstaande is die presiese meganisme nog onseker as gevolg van teenstrydige navorsingsresultate in verskillende etniese groepe. Verskeie onlangse ondersoeke het hipokortisolisme met beide verstedeliking en ’n gevolglike verhoogde moontlikheid van hipertensie aan Afrika vrouens gekoppel omrede hul verhoogde vaskulêre bloeddrukresponse getoon het. Kaukasiër vroue daarteenoor vertoon verhoogde sentrale kardiale response. ’n Gebrek aan data in verband met die voorgenoemde faktore in Afrika- en Kaukasiër vroue, dien as motivering vir die uitvoer van hierdie studie.

Doelstelling: Om die bydrae van hs-CRP, kortisol en hipertensie in die verhoogde moontlikheid van kardiovaskulêre disfunksie in Afrika- en Kaukasiër vroue in Suid Afrika te ondersoek.

Metodologie: Die manuskrip wat in Hoofstuk 2 vervat is, het gebruik gemaak van data wat versamel is tydens die POWIRS (Profiles of Obese Women with Insulin Resistance Syndrome) studie. Oëskynlik gesonde Afrika- (N=102) en Kaukasiër (N=115) vroue, afgepaar vir ouderdom en liggaamsmassa-indeks, is gewerf vir deelname aan die studie in die Noordwes Provinsie van Suid-Afrika. Proefpersone is verdeel in normotensiewe (NT-) en hipertensiewe (HT-) groepe volgens die rustende kardiovaskulêre metings bereken vanaf Finometerwaarnemings. Hoë-sensitiwiteit C-reaktiewe proteïen (hs-CRP) en kortisol bloedserumwaardes is bereken deur immunochemiese en ELISA analises.. Betekenisvolle verskille tussen die etniese groepe en tussen die NT- en HT-groepe is bepaal deur middel van ko-variansie analises (ANCOVA), vir antropometriese, kardiovaskulêre, hs-CRP en kortisolveranderlikes, terwyl daar vir kardiovaskulêre risikofaktore (ouderdom, rook en alkoholverbruik) gekorrigeer is.

Parsiële korrelasies gekorrigeer vir kardiovaskulêre risikofaktore (ouderdom, rook en alkoholverbruik) is uitgevoer om die verhouding tussen hs-CRP, kortisol, antropometrie en kardiovaskulêre veranderlikes te bepaal. Logistiese regressie analises is gebruik in elke etniese groep om die verhouding tussen antropometriese, kardiovaskulêre, hs-CRP en kortisol as onafhanklike veranderlikes en hipertensie as afhanklike veranderlike te bepaal.

Die studie is goedgekeur deur die Etiekkomitee van die Noordwes Universiteit en al die proefpersone het skriftelike toestemming gegee. Vir ’n meer breedvoerige bespreking van die proefpersone, studie-ontwerp en analitiese prosedures wat gevolg is in hierdie verhandeling, word die leser verwys na die Materiale en Metodes afdeling in Hoofstuk 2.

Resultate en Gevolgtrekking: Beide etniese groepe het hoër hs-CRP en laer kortisolwaardes getoon in vergelyking met normaalwaardes. Laer middelomtrek- en kortisolwaardes sowel as hoër bloeddruk (BD) veral vaskulêre waardes is waargeneem in Afrika-vroue in vergelyking met Kaukasiërvroue.

Beide HT etniese groepe was ouer en visseraal meer obees in vergelyking met hul NT eweknie. HT Kaukasiër het hoër sentrale adrenergiese response getoon terwyl HT Afrika-vroue hoër vaskulêre adrenerge response getoon het. Slegs NT Afrika-vroue het laer kortisolwaardes gehad ten opsigte van NT Kaukasiërs maar die Afrikane (NT en HT) het gereageer met hoër diastoliese bloeddrukresponse in vergelyking met hul Kaukasiër eweknie.

Bykomend is hs-CRP in die Afrika-vroue betekenisvol geassosieer met alle BD en obesiteitsveranderlikes terwyl hs-CRP in die HT Kaukasiër vroue slegs geassosieer is met slagvolume (SV) en meegewendheid (Cw). Kortisol in beide etniese groepe is sterk geassosieer met vaskulêre BD reaksies. Slegs BD het sterk bygedrae tot die voorkoms van hipertensie in beide etniese groepe.

er afsluiting, hierdie resultate suggereer die moontlike diverse rol van HPA aksis wanregulering in assosiasie met hoër inflammatoriese response. Dit gebeur ook tergelykertyd in meer obese Kaukasiër en veral Afrika-vroue met respektiewelik, kardiale en vaskulêre response.

Sleutelwoorde: C-Reaktiewe Proteïen, kortisol, hipertensie, obesiteit, kardiovaskulêre disfunksie, Afrika-vroue, Kaukasiërs.

TITLE: The relationship between cortisol, C-reactive protein and hypertension in the development of cardiovascular dysfunction in African and Caucasian women: the POWIRS Study.

SUMMARY

Motivation: C-reactive protein (hs-CRP) and other risk factors such as cortisol and obesity in the diagnosis of cardiovascular dysfunction (CVD) in African and Caucasian women has become increasingly imperative when one considers the escalation of hypertension among these groups. Recent studies have explored some aspects of these risk factors and the roles that they play within hypertension and possible future risk for cardiovascular disease. Hs-CRP has been associated with the increased prevalence of hypertension and obesity. Cortisol *per se* has also been linked with the development of both hypertension and the hypothalamic-pituitary adrenal cortex (HPA) response. Nevertheless, the exact mechanism remains rather uncertain due to conflicting outcomes of research within different ethnic groups. Several recent investigations have, however, linked hypocortisolism with both urbanisation and a subsequent increased likelihood of hypertension within African women as they have presented increased vascular blood pressure responses. Conversely, Caucasian women have displayed an increased central cardiac reactivity. The lack of data regarding the relationship between the above-mentioned parameters within both African and Caucasian women serves as the motivation for conducting this study.

Objective: To investigate hs-CRP, cortisol and hypertension as contributors to the increased likelihood of cardiovascular dysfunction in both African and Caucasian women within South Africa.

hs-CRP use this through whole document please

Methodology: The manuscript presented in Chapter 2 has been compiled using data obtained from the POWIRS (Profiles of Obese Women with Insulin Resistance Syndrome) study. Apparently healthy African (N=102) and Caucasian (N=115) women, matched for age and body mass index, were recruited from the North-West Province of South Africa for participation within this study. Subjects were divided into normotensive (NT) and hypertensive (HT) groups according to the mean resting cardiovascular values that were taken using a Finometer device. High-sensitivity C-reactive protein (hs-CRP) and cortisol blood serum values were determined by immunochemistry and ELISA analyses. Significant differences within each ethnic group and between each of the groups (NT and HT) were determined by analysis of covariance (ANCOVA), for anthropometric, cardiovascular, hs-CRP and cortisol variables, while adjusting for cardiovascular covariates (age, smoking and alcohol consumption). Partial correlations analyses were used to examine the relationship between hs-CRP, cortisol, anthropometric and cardiovascular parameters adjusting for cardiovascular covariates. Logistic regression analyses was used within each ethnic group to determine the relationship between anthropometric, cardiovascular, hs-CRP and cortisol as independent variables and hypertension as dependent variable.

This study was approved by the Ethics Committee of the North-West University and all subjects gave informed consent in writing. For a more detailed description of the subjects, study design and analytical procedures please refer to the Materials and Methods section within Chapter 2 of this dissertation.

Results and Conclusion: Both ethnic groups presented higher hs-CRP and lower cortisol levels compared to normal values. Lower waist circumference (WC) and cortisol as well as higher blood pressure (BP) and vascular values were evident in Africans compared to Caucasians. Both HT ethnic groups were older and more visceral obese compared to their NT counterparts. HT Caucasians indicated higher central adrenergic responses whilst HT Africans showed vascular adrenergicresponses. Only NT Africans had lower cortisol values than NT Caucasians but the Africans (NT and HT) responded with higher diastolic blood pressure responses compared to their Caucasian counterparts. Moreover, hs-CRP within African women significantly correlated with all BP and obesity variables whilst hs-CRP only associated with stroke volume (SV) and compliance (Cw) within HT Caucasian women. Cortisol in both ethnic groups was strongly associated with vascular BP responses. Only BP contributed to the higher prevalence of HT in both ethnic groups.

In conclusion, these results suggest the possible diverse roles of HPA axis dysregulation associated with higher inflammatory responses. This happens in conjunction with cardiac and vascular responses within more obese Caucasian and especially African women, respectively.

Keywords: C-reactive protein, cortisol, hypertension, obesity, cardiovascular dysfunction, Africans, Caucasians.

PREFACE

For the structure of this study it was decided to use the manuscript format. Chapter 1 serves as an introduction and provides the motivation, background and a brief summary of the knowledge necessary for meaningful interpretation of the data. At the beginning of Chapter 2 (the manuscript) is a brief summary of the Instructions for Authors of the peer reviewed journal aimed for publication (the *Journal of Human Hypertension*). Chapter 3 provides a summary of the study results, as well as recommendations for future research. Relevant references are provided at the end of each chapter. The relevant references used in the unpublished Chapters 1 and 3 are provided according to the mandatory style stipulated by the North-West University, Potchefstroom Campus, Potchefstroom, South Africa. The technical style used in Chapters 1 and 3 is, therefore, uniform but differs from Chapter 2 according to the authors' instructions of the specified journal.

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LIST OF ABBREVIATIONS

ANCOVA: Analysis of covariance

SD: standard deviation

CI: confidence interval

CVD: Cardiovascular dysfunction

CCRP: C-reactive protein

hs-CRP: High-sensitivity C-reactive protein

IL-6: interleukin-6

BP: blood pressure

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

HT: hypertensive/hypertension

NT: normotensive/normotension

SV: Stroke volume

CO: Cardiac output

TPR: Total peripheral resistance

Cw: Windkessel compliance

HR: heart rate

WC: waist circumference

BMI: body: mass index

WHR: waist: hip ratio

WHO: World Health Organisation

POWIRS: Profiles of Obese women with Insulin Resistance Syndrome

HPA: Hypothalamic-pituitary-adrenal

CHAPTER 1

INTRODUCTION AND LITERATURE STUDY

Introduction

Links between C-Reactive Protein (CRP) and cardiovascular risk have, of late, lead to a new diagnostic age in which various cardiovascular risk predictors are being utilised in the diagnosis of cardiovascular dysfunction (CVD). More importantly, research pertaining to the use of CRP and other risk factors such as cortisol and obesity in the diagnosis of CVD in African and Caucasian women has become increasingly imperative when one considers the escalation of hypertension among these groups (Opie & Seedat, 2005). Subsequently, it is rather alarming to find that little research has been conducted on these risk factors and their relationship to CVD incidence within these groups. It is therefore of vital importance that research is conducted on the relationship between these factors in both African and Caucasian women in order to establish some fundamentals from which further investigations may be carried out.

Background: CRP and Cortisol

C-reactive protein (CRP), a marker of inflammation, is regulated by interleukin-6 (IL-6) and other cytokines and is produced mainly in the hepatocytes. CRP concentrations, unlike those of IL-6, are relatively constant with regarding circadian rhythm and exhibit relatively stable levels in individuals. Individuals with CRP levels between 1.0 to 3.0 mg/L are classified at moderate risk for cardiovascular disease whereas high-risk individuals are classified as those with CRP levels greater than 3.0mg/L (Labarrere & Zaloga, 2004).

Cortisol is a glucocorticoid hormone that is regulated by the hypothalamic centres that receive stimulatory signals from the central nervous system. The adrenergic,

dopaminergic and serotoninergic systems subsequently modify and regulate these signals resulting in a diurnal secretion of cortisol with high activity occurring in the early morning hours followed by low activity in the afternoon and early evening.[3]

Both CRP and cortisol have been independently associated with hypertension and have been shown to produce hypertension via several mechanisms including those that cause a change in endothelial, sympathetic and/or renal function (Labarrere & Zaloga, 2004; al' Absi *et al.*, 2000; Whitworth *et al.*, 1995; Kullo *et al.*, 2003; Srikumar *et al.*, 2002; Sesso *et al.*, 2003).

CRP, Cortisol and Hypertension

Hypertension can be defined as a chronically increased systolic pressure of 140mmHg or higher in concurrence with a chronically increased diastolic blood pressure of 90mmHg or higher (WHO World Health Organisation, 2003).

An increased relative risk for hypertension in both Africans and African-Americans has been consistently shown, with these individuals often showing higher cardiovascular reactivity measurements than other ethnic groups when a specific stressor was applied (Malan *et al.*, 1992; Van Rooyen *et al.*, 2000; Gerin *et al.*, 2000; Suarez *et al.*, 2004; Knox *et al.*, 2002; Liao *et al.*, 2004; Van Rooyen *et al.*, 2002). Urbanisation factors seem to play a significant role in the higher prevalence of hypertension and/or cardiovascular reactivity in South African Blacks (Van Rooyen *et al.*, 2000; Van Rooyen *et al.*, 2002). This has also been reported within the African-Americans and Caribbean Africans living within the United Kingdom (Siriwardena, 2004). Increases in blood pressure in African-American women have been demonstrated with a simultaneous occurrence of large heart rate increases, peripheral

vasodilation, a greater indexed peripheral resistance (as compared to Caucasians) as well as age-dependent decreases in norepinephrine responses to stress (Suarez *et al.*, 2004; Hinderliter *et al.*, 2004).

CRP levels have been reported to be associated with blood pressure (Kullo *et al.*, 2003; Srikumar *et al.*, 2002; Sesso *et al.*, 2003; de Ferranti & Rifai, 2002; Sung *et al.*, 2003; Libby & Ridker, 2004; Yudkin *et al.*, 2000; Schillaci *et al.*, 2003; Saito *et al.*, 2003; Williams *et al.*, 2004; de Maat & Kluft, 2001). Several studies have demonstrated a significant positive correlation between systolic and diastolic blood pressures and CRP levels (Sung *et al.*, 2003; Yudkin *et al.*, 2000; Schillaci *et al.*, 2003; Saito *et al.*, 2003; Williams *et al.*, 2004; de Maat & Kluft, 2001). Elevated CRP levels appear to be affiliated with hypertension with greater associations occurring among women and in black ethnic groups (Kullo *et al.*, 2003; Srikumar *et al.*, 2002; Sesso *et al.*, 2003; de Ferranti & Rifai, 2002; Sung *et al.*, 2003; Libby & Ridker, 2004; Yudkin *et al.*, 2000; Schillaci *et al.*, 2003; Saito *et al.*, 2003; Williams *et al.*, 2004; de Maat & Kluft, 2001; Kraus *et al.*, 2007). When compared to other markers of inflammation, high-sensitivity C-Reactive Protein (hs-CRP) has been shown to be the strongest predictor of cardiovascular disease in women (Ridker *et al.*, 2000). Ethnic differences in CRP levels have been consistently demonstrated (Albert *et al.*, 2004; Ford *et al.*, 2004; Anand *et al.*, 2004; Wener *et al.*, 2000). Albert *et al.* (2004) reported that CRP levels were significantly higher among African-American women than among their Caucasian, Hispanic and Asian counterparts, even after controlling for age and estrogen use. Another study found that 63% of all African-American women subjects had elevated hsCRP levels (above 3mg/dl) indicating a higher cardiovascular risk amongst this population group (Ben-Yehuda, 2007). Moreover, increased CRP levels have also been associated with increased prevalence of

hypertension amongst United States adults weighing more than 136 kilograms (Mondolfi *et al.,* 2007). Furthermore, Shankar *et al* (2007) stated that higher CRP levels have been associated with peripheral arterial disease amongst United States adults independent of smoking, waist circumference, body mass index, hypertension and other confounders.

Subsequently, it can be deduced that those individuals with higher CRP levels have a greater risk of the development of hypertension and consequently, the development of cardiovascular disease (Labarrere & Zaloga, 2004; Sesso *et al.*, 2003; de Ferranti & Rifai, 2002; Sung *et al.*, 2003; Schillaci *et al.*, 2003; Saito *et al.*, 2003; Williams *et al.*, 2004).

A possible explanation of the development of hypertension due to elevated CRP levels can be explained by considering the relationship between CRP and endothelial damage or dysfunction. Increased CRP levels have been indicated in the elevation of levels of cell adhesion molecules and tissue factor by the activation of the endothelium (Labarrere & Zaloga, 2004; Libby & Ridker, 2004). Subsequently, CRP also induces monocyte recruitment into the arterial intima by heightening the levels of monocyte chemoattractant protein-1(Labarrere & Zaloga, 2004; Libby & Ridker, 2004). The monocytes, in turn, become macrophages once in the intima thereby mediating the uptake of low-density lipoprotein cholesterol (LDL-C) into the endothelium. Foam cells produced by this process in turn cause further release of CRP, leading to further inflammation and consequently, further endothelial damage (Labarrere & Zaloga, 2004). CRP is also known to inhibit nitric oxide and prostacyclin actions on arterial endothelial cells, which subsequently leads to the destruction of endothelial functions such as vasodilation, antithrombogenesis, and finally, antiatherosclerosis (Labarrere & Zaloga, 2004 Sung *et al.*, 2003). It is

therefore believed that chronic inflammation or infection can possibly cause endothelial dysfunction which can subsequently lead to hypertension and cardiovascular diseases.

Elevated cortisol levels are congruous to an activated hypothalamic-pituitary adrenal (HPA) axis that has been stress-induced (al' Absi & Arnett, 2000; Malan *et al.*, 1996; Björntorp, 2001). Furthermore, elevated cortisol levels seem to contribute to the possible role of cortisol in the development of hypertension (al' Absi & Arnett, 2000; Whitworth *et al.*, 1995; Malan *et al.*, 1996; Björntorp, 2001; Kopp & Réthelyi, 2004; Black, 2003). In this regard, Whitworth *et al.* (1995) discussed several mechanisms for cortisol-induced hypertension, which included the following: sodium retention, haemodynamic changes, vascular responsiveness, increased sympathetic nervous activity and hyperinsulinaemia. However, it is still uncertain whether these changes adequately depict all possible causes of cortisol-induced hypertension as more recent studies have found that hypocortisolism has in some instances been related to hypertension and/or CVD risk factors (Kopp & Réthelyi, 2004; Heim *et al.*, 2000; Huisman *et al.*, 2002). One such study by Huisman *et al.* (2002) included a sample of urbanized African women who were found to display decreased cortisol levels in concurrence with a corresponding increased incidence of hypertension.

CRP, mediated by interleukin-6 (IL-6) has been shown to increase during an inflammatory reaction (Black & Garbutt, 2002). Black & Garbutt (2002) postulate that since the inflammatory response is contained within the stress response, such a response to a stressor induces a corresponding stress/inflammatory response in the arteries, that if repetitive or chronic, may ultimately lead to hypertension and/or atherosclerosis. Essentially the above-mentioned postulate suggests that the so-called

"stress hormones", which include cortisol, actually initiate a response that produces acute phase reactants such as CRP similar to that of an inflammatory reaction within the arteries which will consequently lead to hypertension.

CRP, mediated by IL-6, has been shown to correlate with increased cortisol release (Steensburg *et al.*, 2003; Boss & Neeck, 2000; Girod & Brotman, 2004). However, in some clinical settings, short-term exposure to glucocorticoids, such as cortisol, has been associated with decreased CRP levels (Girod & Brotman, 2004; Kunz-Ebrecht *et al.*, 2003). Nonetheless, longer exposure to cortisol seems to produce increased levels of CRP (Girod & Brotman, 2004; Kunz-Ebrecht *et al.*, 2003). One possible explanation of the above-mentioned findings includes the release of IL-6, and subsequently, CRP, from different sites according to the length of cortisol exposure (Girod & Brotman, 2004). Other possible explanations include genetic predisposition to certain types of release at the genetic transcription level (Girod & Brotman, 2004) as well as gender-specific differential effects on the immune system modulation of cortisol production and consequently, CRP release (Kopp & Réthelyi, 2004). Notwithstanding, the current most prevalent findings suggest HPA axis dysregulation according to contrasting types of stress as a likely explanation for the noted discrepancies in the cortisol and CRP interrelationships mentioned previously (Kopp & Réthelyi, 2004; Black, 2003; Heim *et al.*, 2000; Kunz-Ebrecht *et al.*, 2003). Regardless of the above-mentioned findings, the current understanding of these relationships within different ethnic groups has, as of yet, been neglected.

CRP, Cortisol and Obesity

Obesity can be defined as the storage of excess calories in fat. It can be measured in various ways including percent of body fat, skin-fold thicknesses, waist: hip circumference ratio (WHR) and body mass index (BMI). A BMI of 30 or greater is considered to be an indication of obesity (Björntorp &Brodoff, 1992; World Helath Organization, 1997).

There has been an increased prevalence of obesity over the past few decades with a higher predominance occurring in women (Mufunda *et al.*, 2000; Rexrode *et al.*, 2003; Das, 2001). One particular study in which the Zimbabwean population was investigated for correlates of blood pressure found that the average BMI for women within this group fell within the overweight range and that most of the anthropometric variables, including BMI, peaked or plateaued in the 35-44 year age group (Mufunda *et al.*, 2000).

Several measures of obesity, including body mass index (BMI), waist: hip ratio (WHR) and percentage body fat (% BF) have relationships with CRP levels (Kullo *et al.*, 2003; Saito *et al.*, 2003; de Maat & Kluft, 2001; Rexrode *et al.*, 2003; Das, 2001; Forouhi *et al.*, 2001; Ford, 1999; Tucci *et al.*, 2003; Saijo *et al.*, 2004). Elevated CRP levels have been consistently associated with increasing BMI, waist circumference (WC), WHR, % BF, triceps skinfold thicknesses and visceral fat area, among other indicators of body fat and obesity (Kullo *et al.*, 2003; Rexrode *et al.*, 2003; Das, 2001; Ford, 1999; Vikram *et al.*, 2003). The above-mentioned indicators of body fat and obesity have also been evidenced to be significantly correlated with CRP levels (Saito *et al.*, 2003; Williams *et al.*, 2004; Rexrode *et al.*, 2003; Forouhi *et al.*, 2001; Tucci *et al.*, 2003; Saijo *et al.*, 2004; Vikram *et al.*, 2003; Lear *et al.*, 2003). One such study

demonstrated that obesity was independently related to CRP in women (Williams *et al.*, 2004). CRP has been shown to have its strongest correlations with BMI and WC and weaker correlations with % BF, triceps skinfold thickness and WHR (Rexrode *et al.*, 2003; Vikram *et al.*, 2003; Lear *et al.*, 2003). The association between CRP and various measures of obesity has been shown to be significantly stronger in European, Chinese and Indian women than in their male counterparts (Williams *et al.*, 2004; Vikram *et al.*, 2003; Lear *et al.*, 2003). These associations have also been shown to differ among ethnic groups (Forouhi *et al.*, 2001; Lear *et al.*, 2003; Schutte *et al.*, 2006; Schutte *et al.*, 2008). Albert *et al.* (2004) also reported that BMI was a significant confounder of CRP levels in all women; however, this effect was most noticeable amongst African-American women. As mentioned previously significant differences between genders have been reported regarding their respective CRP levels (Kullo *et al.*, 2003; Saito *et al.*, 2003; Williams *et al.*, 2004; Vikram *et al.*, 2003). Women tend to have higher CRP levels than their male counterparts with a few exceptions (Kullo *et al.*, 2003; Saito *et al.*, 2003; Williams *et al.*, 2004; Vikram *et al.*, 2003). However, these exceptions have a tendency to occur in the Asian, Indian and Japanese populations where overall mean BMI levels for corresponding ages tend to be lower than for other populations (Saito *et al.*, 2003; Vikram *et al.*, 2003). Lear *et al.* (2003) provide a possible explanation of the higher observed CRP levels in women by stating that women have a higher percent of body fat compared with men at any given BMI.

A plausible mechanism that could explain the elevated CRP levels that are associated with increases in body fat could possibly be ascribed to the increased expression of IL-6 in adipose tissues (Das, 2001; Yudkin *et al.*, 2000). As mentioned previously, IL-6 is a proinflammatory cytokine that is released into the circulation which in turn

stimulates the production of CRP in the liver. The production of IL-6 has been shown to increase with increasing adiposity in healthy men and women (Yudkin *et al.*, 2000). The expression of IL-6 in adipose tissue has been thought to have a greater contribution from visceral than subcutaneous fat (Misra & Vikram, 2003; Frühbeck & Salvador, 2004). This could possibly explain the higher correlations of CRP with visceral fat indicators such as waist circumference. Thus, in summary, higher IL-6 concentrations produced as a result of higher adiposity could possibly lead to the higher CRP levels observed with obesity.

Cortisol has been positively correlated with obesity and has been shown to have increased levels in obese individuals (Björntorp & Rosmond, 2000; Björntorp, 2001). Cortisol levels have also been shown to increase significantly with increasing BMI and percentage body fat (Purnell *et al.*, 2004). Since cortisol binds to glucocorticoid receptors, it is found that there is a higher accumulation of cortisol within the visceral fat depots within the body as the highest density of these receptors occurs within the visceral adipose depots (Björntorp, 2001). Thus the activity of cortisol-induced accumulation of fat will also be heightened within this particular adipose tissue depots.[32] Furthermore, WHR and WC measurements, which are more accurate measures of abdominal and/or visceral obesity, seem to correlate far more highly with cortisol than those of BMI and other indicators of overall obesity (Björntorp, 2001; Misra & Vikram, 2003). Cortisol secretion due to increased adiposity or obesity has been suggested to occur via the dysregulation of the HPA axis (Björntorp & Rosmond, 2000; Björntorp, 2001). Since increased cortisol levels leads to an increase in abdominal adipose tissue stores and subsequently higher likelihood of obesity, one can hypothesise that increased cortisol will lead to an increased probability of cardiovascular dysfunction (Björntorp, 2001). One such plausible mechanism

includes elevation of cortisol due to over-activity of the HPA axis which in turn contributes to increased accumulation of abdominal or visceral adiposity and finally increased fatty acid levels in the bloodstream, following the release of these high levels of fat, which subsequently could lead to increased cardiovascular risk.

Other products that are secreted by adipose tissue include IL-6, which is a mediator of CRP release (Black, 2003; Misra & Vikram, 2003; Frühbeck & Salvador, 2004; Ahima & Flier, 2000). Moreover, IL-6 expression occurs in higher magnitudes within visceral adipose tissue in comparison to subcutaneous adipose tissue (Black, 2003; Misra & Vikram, 2003). Furthermore CRP production has also been correlated to HPA axis function (Kopp & Réthelyi, 2004; Black, 2003; Heim *et al.*, 2000; Huisman *et al.*, 2002). The abnormal activity or dysregulation of the HPA axis may lead to the over-production of CRP (Kopp & Réthelyi, 2004; Black, 2003). Hypercortisolism due to HPA axis dysregulation has been shown to occur in concurrence with increased CRP production (Kopp & Réthelyi, 2004; Black, 2003). Hence, both increased cortisol and CRP production normally occur in conjunction with both abnormal HPA axis activity and obesity, (Kopp & Réthelyi, 2004; Black, 2003) and subsequently, the higher likelihood of hypertension and cardiovascular dysfunction.

Cardiovascular Disease: Other risk factors

Several factors have been established as possible risk factors for the increased likelihood of cardiovascular disease (CVD). Some of these include a sedentary lifestyle or overweight/obese subjects, especially those with central obesity; a family history of premature CVD; ethnicity; gender; those individuals with a low socio-economic status and, lastly, diabetes (five-fold higher risk in women for CVD)

(Graham *et al.*, 2007). An increased risk of coronary heart disease (CHD) and other adverse health affects have also been demonstrated in those individuals who passively smoke (Graham *et al.*, 2007). These risk factors are especially of importance when one has to consider likely variables to adjust for when conducting cardiovascular risk studies.

CRP, Cortisol, Obesity and Cardiovascular Disease: The South African Perspective

As mentioned previously one has to take into account the influence of different ethnic groups and the distinct differences in both cardiovascular and other markers on the likelihood of cardiovascular disease within these groups. Several studies within Southern Africa have already taken some of these factors into account when exploring these relationships. These factors include the influence of urbanisation, CRP and cortisol on the prevalence of hypertension within different ethnic groups (Van Rooyen *et al.*, 2000; Van Rooyen *et al.*, 2002; Huisman *et al.*, 2002; Seedat, 1998; Schutte *et al.*, 2006; Schutte *et al.*, 2008). Urbanisation has been shown to play a significant role in increasing cardiovascular reactivity within Africans when compared to other ethnic groups (Van Rooyen *et al.*, 2000; Van Rooyen *et al.*, 2002; Seedat, 1998). These studies have also demonstrated a corresponding higher prevalence of hypertension amongst this group when compared with Caucasians, Indians and those of mixed origin (Van Rooyen *et al.*, 2000; Van Rooyen *et al.*, 2002; Seedat, 1998). African women have previously been shown to have significantly higher hsCRP levels and BP readings when compared with their Caucasian counterparts however no cardiovascular parameters could explain the variation in this inflammatory marker

(Schutte *et al.*, 2006). Caucasian women, however, did show strong significant correlations with Cw and TPR (Schutte *et al.*, 2006). Nevertheless these correlations became non-significant and weak after adjustments were made for age, BMI and WC (Schutte *et al.*, 2006). Another recent study by Schutte *et al* (2008) has demonstrated yet again that African women had higher BP and HT rates in conjunction with higher arterial resistance and lower CO as compared to their Caucasian counterparts. The exact mechanism of this increased prevalence of hypertension amongst this ethnic group is still unknown but great strides have been made in the determination of other possible risk factors. Hypocortisolism, in conjunction with urbanisation, has been proposed as a possible contributing factor to the increased incidence of hypertension within African women (Huisman *et al.*, 2002), however further studies need to be conducted in order to further assess what role this observation plays in association with other risk factors for CVD within this ethnic group.

Furthermore, one has to take into account the roles of obesity on the abovementioned CVD risk within these ethnic groups in Southern Africa if one is investigating possible risk factors for future CVD. Schutte *et al* (2008) recently investigated whether the high prevalence of hypertension within South African women could be attributed to obesity and found that although urbanised African women have higher BP than Caucasian women, their obesity levels were more weakly correlated to traditional risk factors (specifically SBP, arterial resistance and CO) when compared to their counterparts. Moreover, these markers showed no significant difference between both low and high BP African groups. CRP was one of the inflammatory markers that displayed a significant correlation with obesity within African women during this study. Another study confirmed the above-mentioned observations, however, once adjustments for age and obesity (BMI and WC) were made, these

correlations became insignificant (Schutte *et al.*, 2006). Both these studies thus suggest that perhaps obesity should not be considered as a cardiovascular risk within African women, when compared to Caucasian women (Schutte *et al.*, 2006; Schutte *et al.*, 2008). Rather, it is proposed that since there is a strong correlation between markers of type II diabetes and obesity within African women, that the possible mechanism of cardiovascular risk could be attributed secondarily to the initial effect of obesity on the development of diabetes which, in turn, could lead to CVD (Schutte *et al.*, 2008).

In conclusion, acute or chronic stress may lead to dysregulation of the HPA axis which in turn is mediated by certain inflammatory markers such as CRP and other stress hormones which include cortisol. Free fatty acids which are released from increased visceral or abdominal adipose tissues also play a role in heightening levels of CRP. Sequentially, heightened levels of cortisol are also linked to increased adiposity and subsequently increased inflammation, which consequently leads to increased cardiovascular disease risk. Moreover, this linkage is further supported by the decrease in inflammatory mediators in individuals where weight loss was demonstrated (Black, 2006).

It is important to note however that although several of the above-mentioned studies have explored this relationship in detail, and while guidelines have been set for other ethnic groups (Black, 2006), the investigation of African and Caucasian women from South Africa has been limited. It is thus imperative that these relationships be explored further within these ethnic groups within this country in order to fully substantiate and assess possible risk for CVD within these groups. This in turn, may subsequently assist researchers to isolate possible mechanisms of action of hypertension within these groups and accordingly allow for better clinical approaches

to this disease which may consequently reduce its potential to influence future cardiovascular health negatively.

Research Question

Although a number of studies have investigated the roles of CRP, cortisol and hypertension and their roles within cardiovascular disease, it seems that there exists a need to further investigate these factors within both African and Caucasian women in South Africa. This chapter indicated that both increased CRP, and decreased cortisol, in conjunction with hypertension may lead to a higher risk for cardiovascular disease within these groups.

Aim

The aim of this study is to investigate CRP, cortisol and hypertension as contributors to the increased likelihood of cardiovascular disease in both African and Caucasian women from South Africa.

Hypotheses

1. African women, compared to Caucasian women will present with higher CRP levels and subsequently, positive associations with hypertension.
2. African women, compared to Caucasian women will present with lower cortisol levels and subsequently, positive associations with elevated vascular responses and consequently, hypertension.

REFERENCES

AHIMA, R.S. & FLIER, J.S. 2000. Adipose tissue as an endocrine organ. *TEM*, 11(8): 327-332.

AL' ABSI, M. & ARNETT, D.K. 2000. Adrenocorticol responses to psychological stress and risk for hypertension. *Biomed & Pharmacother*, 54: 234-244.

ALBERT, M.A., GLYNN, R.J., BURING, J. & RIDKER PM. 2004. C-reactive protein levels among women of various ethnic groups living in the United States (from the Women's Health Study). *Am J Cardiol*, 93(10): 1238-1242.

ANAND, S.S., RAZAK, F., YI, Q., DAVIS, B., JACOBS, R., VUKSAN, V., LONN, E., TEO, K., MCQUEEN, M. & YUSUF, S. 2004. C-reactive protein as a screening test for cardiovascular risk in a multiethnic population. *Arterioscler Thromb Vasc Biol*, 24: 1509.

BEN-YEHUDA, O. 2007. High-sensitivity C-reactive protein in every chart? *JACC*, 49(21): 2139-2141.

BJÖRNTORP, P. & BRODOFF, B.N. 1992. Obesity. Lippincott: Pennsylvania. 805pp.

BJÖRNTORP, P. & ROSMOND R. 2000. *Obesity and cortisol*, Nutrition, 16(10): 924-936.

BJÖRNTORP, P. 2001. Do stress reactions cause abdominal obesity and comorbidities? *Obes Rev*, 2: 73-86.

BLACK, P.H. & GARBUTT L.D. 2002. Stress, inflammation and cardiovascular disease. *J Psychosom Res*, 52: 1-23.

BLACK, P.H. 2006. The inflammatory consequences of psychologic stress: Relationship to insulin resistance, obesity, atherosclerosis and diabetes mellitus, type II. *Med Hypotheses*, 67: 879-891.

BLACK, P.H. 2003. The inflammatory response is an integral part of the stress response: Implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X. *Brain Behav Immun*, 17: 350-364.

BOSS B. & NEECK, G. 2000. Correlation of IL-6 with the classical humoral disease activity parameters ESR and CRP and with serum cortisol, reflecting the activity of the HPA axis in active rheumatoid arthritis. *Z Rheumatol*, 59(2): 1162-1164.

DAS, U.N. 2001. Is obesity an inflammatory condition? *Nutrition*, 17: 953-966.

DE FERRANTI, S. & RIFAI, N. 2002. C-reactive protein and cardiovascular disease : a review of risk prediction and interventions. *Clin Chim Acta*, 317: 1-15.

DE MAAT, M.P. & KLUFT, C. 2001. Determinants of C-reactive protein concentration in blood. *Ital Heart J*, 2(3): 189-195.

FORD, E.S. 1999. Body mass index, diabetes, and C-reactive protein among U.S. adults. *Diabetes Care*, 22(12): 1971-1977.

FORD, E.S., GILES, W.H., MOKDAD, A.H. & MYERS, G.L. 2004. Distribution and correlates of C-reactive protein concentrations among adult US women. *Clin Chem*, 50: 574-581.

FOROUHI, N.G., SATTAR, N. & MCKEIGUE, P.M. 2001. Relation of C-reactive protein to body fat distribution and features of the metabolic syndrome in Europeans and South Asians. *Int J Obes*, 25: 1327-1331.

FRÜBECK. G. & SALVADOR, J. 2004. Role of adipocytokines in metabolism and disease. *Nutrition Research*, 24: 803-826.

GERIN, W., PICKERING, T.G., GLYNN, L., CHRISTENFELD, N., SCHWARTZ, A., CARROLL, D. & DAVIDSON, K. 2000. An historical context for behavioral models of hypertension. *J Psychosom Res*, 48: 369-377.

GIROD, J.P. & BROTMAN, D.J. 2004. Does altered glucocorticoid homeostasis increase cardiovascular risk? *Cardiovasc Res*, 64: 217-226.

GRAHAM, I., ATAR, D., BORCH-JOHNSEN, K., BOYSEN, G., BURELL, G., CIFKOVA, R., DALLONGEVILLE, J., DE BACKER, G., EBRAHIM, S., GJELSVIK, B., HERRMANN-LINGEN, C., HOES, A., HUMPHRIES, S., KNAPTON, M., PERK, J., PRIORI, S., PYORALA, K., REINER, Z., RUILOPE, L.,

SANS-MENDEZ, S., REIMER, W.S.O., WEISSBERG, P., WOOD,D., YARNELL, J. & ZAMORANO, J.L. 2007. European guidelines on cardiovascular disease prevention in clinical practice: Executive summary. *Atherosclerosis*, 194: 1-45.

HEIM, C., EHLERT, U. & HELLHAMMER, D.H. 2000. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychneuroendocrinolgy*, 25: 1-35.

HINDERLITER, A.L., BLUMENTHAL, J.A., WAUGH, R., CHILUKURI, M. & SHERWOOOD, A. 2004. Ethnic differences in left ventricular structure: relations to hemodynamics and diurnal blood pressure variation. *AJH*, 17: 43-49.

HUISMAN, H.W., VAN ROOYEN, J.M., MALAN, N.T., ELOFF, F.C., MALAN, L., LAUBSCHER, P.J. & SCHUTTE, A.E. 2002. Prolactin, testosterone and cortisol as possible markers of changes in cardiovascular function associated with urbanization. *J Hum Hypertens*, 16: 829-835.

KNOX, S.S., HAUSDORFF, J. & MARKOVITZ, J.H. 2002. Reactivity as a predictor of subsequent blood pressure. Racial differences in the coronary artery risk development in young adults (CARDIA) study. *Hypertension*, 40: 914.

KOPP, M.S. & RÉTHELYI, J. 2004. Where psychology meets physiology: chromic stress and premature mortality - the Central-Eastern European health paradox. *Brain Res Bull*, 62: 351-367.

KRAUS, V.B., STABLER, T.V., LUTA, G., RENNER, J.B., DRAGOMIR, A.D. & JORDAN, J.M. 2007. Interpretation of serum C-reactive protein (CRP) levels for cardiovascular disease risk is complicated by race, pulmonary disease, body mass index, gender, osteoarthritis. *Osteoarthritis Cartilage*, 15: 966-971.

KULLO, I.J., MCCONNNELL, J.P., BAILEY, K.R., KARDIA, S.L., BIELAK, L.F., PEYSER, P.A., SHEEDY, P.F., BOERWINKLE, E. & TURNER, S.T. 2003. Relation of C-reactive protein and fibrinogen to coronary artery calcium in subjects with systemic hypertension. *Am J Cardiol*, 92: 56-58.

KUNZ-EBRECHT, S.R., MOHAMED-ALI, V., FELDMAN, P.J., KIRSCHBAUM, C. & STEPTOE, A. 2003. Cortisol responses to mild psychological stress are inversely associated with proinflammatory cytokines. *Brain Behav Immun*, 17: 373-383.

LABARRERE, C.A. & ZALOGA, G.P. 2004. C-reactive protein: From innocent bystander to pivotal mediator of atherosclerosis. *Am J Med*, 117(7): 499-507.

LEAR, S.A., CHEN, M.M., BIRMINGHAM, C.L. & FROHLICH, J.J. 2003. The relationship between simple anthropometric indices and C-reactive protein: ethnic and gender differences. *Metabolism*, 52(12): 1542-1546.

LIAO, Y., TUCKER, P., OKORO, C.A., GILES, W.H., MOKDAD, A.H. & HARRIS, V.B. 2004. REACH 2010 Surveillance for health status in minority communities-

United States, 2001-2002. *MMWR. Surveillance summaries: Morbidity and mortality weekly report. Surveillance summaries,* 53(6): 1-36.

LIBBY, P. & RIDKER, P.M. 2004. Inflammation and atherosclerosis: Role of C-reactive protein in risk assessment. *Am J Med,* 116(6A): 9S-16S.

MALAN, N.T., VAN DER MERWE, J.S., HUISMAN, H.W., KRUGER, A., ELOFF, F.C., PRETORIUS, P.J. & LAUBSCHER, P.J. 1992. A comparison of cardiovascular reactivity of rural blacks, urban blacks and whites. *Stress Medicine,* 8: 1-6.

MALAN, N.T., BRITS, J.S., ELOFF, F.C., HUISMAN, H.W., KRUGER, A., LAUBSCHER, P.J. & PRETORIUS, P.J. 1996. The influence of acculturation on endocrine reactivity during acute stress in urban black males. *Stress Medicine,* 12: 55-63.

MISRA, A. & VIKRAM, N.K. 2003. Clinical and pathophysiological consequences of abdominal adiposity and abdominal adipose tissue depots. *Nutrition,* 19: 457-466.

MONDOLFI, R.N., JONES, T.M., HYRE, A.D., RAGGI, P. & MUNTNER, P. 2007. Comparison of percent of United States adults weighing $\geqslant$ 300 pounds (136 kilograms) in three time periods and comparison of five atherosclerotic risk factors for those weighing $\geqslant$300 pounds to those <300 pounds. *Am J Cardiol,* 100: 1651-1653.

MUFUNDA, J., SCOTT, L.J., CHIFAMBA, J., MATENGA, J., SPARKS, B., COOPER, R. & SPARKS, H. 2000. Correlates of blood pressure in an urban Zimbabwean population and comparison to other populations of African origin. *J Hum Hypertens*, 14: 65-73.

OPIE, L.H. & SEEDAT, Y.K. 2005. Hypertension in sub-Saharan African populations. *Circulation*, 112: 3562-3568.

OPIE, L.H. 1998. The Heart: Physiology from cell to circulation, 3rd ed. Lippencott/Raven Publishers: Philadelphia. 19-25pp, 276pp, 288pp.

PURNELL, J.Q., BRANDON, D.D., ISABELLE, L.M., LORIAUX, D.L. & SAMUELS, M.H. 2004. Association of 24-hour cortisol production rates, cortisol-binding globulin, and plasma-free cortisol levels with body composition, leptin levels, and aging in adult men and women. *J Clin Endocrinol Metab*, 89(1): 281-287.

REXRODE, K.M., PRADHAN, A., MANSON, J.E., BURING, J.E. & RIDKER, P.M. 2003. Relationship of total and abdominal adiposity with CRP and IL-6 in women. *AEP*, 13(10): 674-682.

RIDKER, P.M., HENNEKENS, C.H., BURING, J.E. & RIFAI, N. 2000. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *The New England Journal of Medicine*, 342: 836-843.

SAIJO, Y., KIYOTA, N., KAWASAKI, Y., MIYAZAKI, Y., KASHIMURA, J., FUKUDA, M. & KISHI, R. 2004. Relationship between C-reactive protein and visceral adipose tissue in healthy Japanese subjects. *Diabetes Obes Metab*, 6(4): 249.

SAITO, M., ISHIMITSU, T., MINAMI, J., ONO, H., OHRUI, M. & MATSUOKA, H. 2003. Relations of plasma high-sensitivity C-reactive protein to traditional cardiovascular risk factors. *Atherosclerosis*, 167: 73-79.

SCHILLACI, G., PIRRO, M., GEMELLI, F., PASQUALINI, L., VAUDO, G., MARCHESI, S., SIEPI, D., BAGAGLIA, F., VITALE, R. & MANNARINO, E. 2003. Systolic and pulse pressure as major culprits of increased plasma C-reactive protein levels in patients with newly diagnosed hypertension. *AJH*, 16(5): 258A.

SCHUTTE, A.E., VAN VUUREN, D., VAN ROOYEN, J.M., HUISMAN, H.W., SCHUTTE, R., MALAN, L. & MALAN, N.T. 2006. Inflammation, obesity and cardiovascular function in African and Caucasian women from South Africa: the POWIRS study. *J Hum Hypertens*, 20: 850-859.

SCHUTTE, A.E., HUISMAN, H.W., VAN ROOYEN, J.M., SCHUTTE, R., MALAN, L., REIMANN, M., DE RIDDER, J.H., VAN DER MERWE, A., SCHWARZ, P.E.H. & MALAN, N.T. 2008. Should obesity be blamed for the high prevalence rates of hypertension in black South African women? *J Hum Hypertens*, 22: 528-536.

SEEDAT, Y.K. 1998. The prevalence of hypertension and the status of cardiovascular health in South Africa. *Ethn Dis,* 8: 394-397.

SESSO, H.D., BURING, J.E., RIFAI, N., BLAKE, G.J., GAZIANO, J.M. & RIDKER, R.M. 2003. C-reactive protein and the risk of developing hypertension. *JAMA*, 290: 2945-2951.

SHANKAR, A., LI, J., NIETO, J., KLEIN, B.E.K. & KLEIN, R. 2007. Association between C-reactive protein level and peripheral arterial disease among US adults without cardiovascular disease, diabetes, or hypertension. *Am Heart J*, 154: 495-501.

SIRIWARDENA, A.N. Specific health issues in ethnic minority groups. 2004. *Clin Cornerstone*, 611: 34-42.

SRIKUMAR, N., HOPKINS, P.N., JEUNEMAITRE, X., HUNT, S.C. & WILLIAMS, G.H. 2002. Inflammatory marker C-reactive protein in hypertensive subjects. *AJH*, 15(4): 227A.

STEENSBURG, A., FISCHER, C.P., KELLER, C., MØLLER, K. & PEDERSEN, B.K. 2003. IL-6 enhances plasma IL-1ra, IL-10 and cortisol in humans. *Am J Physiol Endocrinol Metab*, 48(2): E433.

SUAREZ, E.C., SAAB, P.G., LLABRE, M.M., KUHN, C.M. & ZIMMERMAN, E. 2004. Ethnicity, gender, and age effects on adrenoreceptors and physiological responses to emotional stress. *Psychophysiology*, 41: 450-460.

SUNG, K.C., SUH, J.Y., KIM, B.S., KANG, J.H., KIM, H., LEE, M.H. PARK, J.R. & KIM, S.W. 2003. High sensitivity C-reactive protein as an independent risk factor for essential hypertension. *AJH*, 16: 429-433.

TUCCI, M., DESIDERI, G., MAZZA, A., PASSACQUALE, G., BROCCOLETTI, S., VALERI, L., BONTEMPO, G., STATI, M., CROCE, G., SANTUCCI, A. & FERRI, C. 2003. Obesity and vascular wall inflammation, effects of weight loss and weight cycling. *AJH*, 16(5): 257A.

VAN ROOYEN, J.M., KRUGER, H.S., HUISMAN H.W., WISSING, M.P., MARGETTS, B.M., VENTER, C.S. & VORSTER, H.H. 2000. An epidemiological study of hypertension and its determinants in a population in transition: the THUSA study. *J Hum Hypertens,* 14: 779-787.

VAN ROOYEN, J.M., HUISMAN H.W., ELOFF, F.C., LAUBSCHER, P.J., MALAN, L., STEYN, S. & MALAN, N.T. 2002. Cardiovascular reactivity in black South African males of different age groups: the influence of urbanization. *Ethn Dis,* 12: 69-75.

VIKRAM, N.K., MISRA, A., DWIVEDI, M., SHARMA, R., PANDEY, R.M., LUTHRA, K., CHATTERJEE, A., DHINGRA, V., JAILKHANI, B.L., TALWAR, K.K. & GULERIA, R. 2003. Correlations of C-reactive protein levels with anthropometric profile, percentage of body fat and lipids in healthy adolescents and young adults in urban North India. *Atherosclerosis,* 168: 305-313.

WENER, M.H., DAUM, P.R. & MCQILLAN, G.M. 2000. The influence of age, sex, and race on the upper reference limit of serum C-reactive protein concentration. *J Rheumatol*, 27(10): 2351-2359.

WHITWORTH, J.A., BROWN, M.A., KELLY, J.J. & WILLIAMSON, P.M. 1995. Mechanisms of cortisol-induced hypertension in humans. *Steroids*, 60: 76-80.

WHO (WORLD HEALTH ORGANIZATION). 2003. World Health Organization/ International Society of Hypertension (ISH) statement on management of hypertension. *J Hypertens*, 21: 1983-1992.

WILLIAMS, M.J.A., WILLIAMS, S.M., MILNE, B.J., HANCOX, R.J. & POULTON, R. 2004. Association between C-reactive protein, metabolic cardiovascular risk factors, obesity and oral contraceptive use in young adults. *Int J Obes*, 28: 998-1003.

WORLD HEALTH ORGANIZATION. 1997. Obesity: Preventing and managing the global epidemic. Report of a WHO consultation on obesity, June 3-5, 1997, Geneva, Switzerland. World Health Organisation: Geneva.

YUDKIN, J.S., KUMARI, M., HUMPHRIES, S.E. & MOHAMED-ALI, V. 2000. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis*, 148: 209-214.

CHAPTER 2

THE RELATIONSHIP BETWEEN CORTISOL, C-REACTIVE PROTEIN AND HYPERTENSION IN THE DEVELOPMENT OF CARDIOVASCULAR DYSFUNCTION IN AFRICAN AND CAUCASIAN WOMEN: THE POWIRS STUDY

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Meyer HA. The Role of Abdominal Fat, 2nd edn. Academic Publishers: Dordrecht, 1970, 179pp.

Harley NH, Vivian L. Invading microorganisms. In: Sodeman WA, Smith A (eds). Mechanisms of Disease, 4th edn.

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THE RELATIONSHIP BETWEEN CORTISOL, C-REACTIVE PROTEIN AND HYPERTENSION IN THE DEVELOPMENT OF CARDIOVASCULAR DYSFUNCTION IN AFRICAN AND CAUSCASIAN WOMEN: THE POWIRS STUDY

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Abstract

Research on the roles that both C-reactive protein (CRP) and other risk factors such as cortisol and obesity play within the diagnosis of cardiovascular dysfunction (CVD) in African and Caucasian women has become increasingly imperative when one considers the escalation of hypertension among these groups. CRP has been associated with increased hypertension and obesity. Cortisol has also been linked with both hypertension and the hypothalamic-pituitary adrenal (HPA) response. African women have previously presented with an observed increased vascular reactivity. Conversely, Caucasian women have displayed an increased central cardiac reactivity. Apparently healthy African (N=102) and Caucasian (N=115) women, matched for age and body mass index, were included. Elevated CRP levels were observed within African women when compared to Caucasian women. CRP within African women significantly correlated with total peripheral resistance (TPR), Windkessel compliance (Cw) and systolic blood pressure (SBP) within the hypertensive range. Significant correlations of CRP with both stroke volume (SV) and Cw were demonstrated within Caucasian women within the hypertensive range. A discernable hypocortisolism was exhibited within both ethnic groups during the hypertensive range. African women presented with significant differences between vascular markers Cw and TPR when comparing these markers within the normotensive (NT) and hypertensive (HT) ranges, respectively. Conversely, Caucasian women exhibited significant differences between the cardiac markers SV and cardiac output (CO) when comparing these ranges. These results suggest the possible diverse roles that HPA axis dysregulation can play, in conjunction with the respective cardiac and vascular responses within both Caucasian and African women, in future cardiovascular risk for these groups.

Keywords: C-reactive protein, cortisol, hypertension, obesity, cardiovascular dysfunction, Africans, Caucasians.

Word count: 254

Introduction

Research pertaining to the use of C-reactive protein (CRP) and other risk factors such as cortisol and obesity in the diagnosis of cardiovascular dysfunction (CVD) in African and Caucasian women has become increasingly imperative when one considers the escalation of hypertension among these groups. [1] Subsequently, it is rather alarming to find that little research has been conducted regarding these risk factors and their relationship to CVD incidence within these groups. It is therefore of vital importance that research is conducted into the relationship between these factors in both African and Caucasian women in order to establish some fundamentals from which further investigations can be carried out.

Several studies within Southern Africa have explored the above-mentioned relationships between cardiovascular, anthropometric and other risk factors in possible future CVD. These factors include the influence of urbanisation, CRP and cortisol on the prevalence of hypertension within different ethnic groups.[2-7] Urbanisation has been shown to play a significant role in increasing cardiovascular reactivity within Africans when compared to other ethnic groups.[2,3,5] These studies have also demonstrated a corresponding higher prevalence of hypertension amongst this group when compared with Caucasians, Indians and those of mixed origin.[2,3,5] African women have previously been shown to have significantly higher high-sensitivity C-reactive protein (hsCRP) levels and blood pressure (BP) readings when compared with their Caucasian counterparts. However, no cardiovascular parameters could explain the variation in this inflammatory marker.[6] Caucasian women, however, did show strong significant correlations with Windkessel compliance (Cw) and total

peripheral resistance (TPR).[6] Nevertheless these correlations became non-significant and weak after adjustments were made for age, body:mass index (BMI) and waist circumference (WC).[6] Another recent study by Schutte *et al* [7] has demonstrated yet again that African women had higher BP and hypertension (HT) rates in conjunction with higher arterial resistance and lower cardiac output (CO) as compared to their Caucasian counterparts. The exact mechanism of this increased prevalence of hypertension amongst this ethnic group is still unknown but great strides have been made in the determination of other possible risk factors. Hypocortisolism, in conjunction with urbanisation, has been proposed as a possible contributing factor to the increased incidence of hypertension within African women,[4] however further studies need be conducted in order to further assess what role this observation plays in association with other risk factors for CVD within this ethnic group.

Furthermore, one has to take into account the roles of obesity on the above-mentioned CVD risk within these ethnic groups in Southern Africa if one is investigating possible risk factors for future CVD. Schutte *et al* [7] recently investigated whether the high prevalence of hypertension within South African women could be attributed to obesity and found that although urbanised African women have higher BP than Caucasian women, their obesity levels were more weakly correlated to traditional risk factors (specifically systolic blood pressure, SBP, arterial resistance and CO) when compared to their counterparts. Moreover, these markers showed no significant difference between both low and high-BP African groups. CRP was one of the inflammatory markers that displayed a significant correlation with obesity within African women during this study. Another study confirmed the above-mentioned observations. However, once adjustments for age and obesity (BMI and WC) were made, these correlations became insignificant.[6] Both these studies thus suggest that

perhaps obesity should not be considered as cardiovascular risk within African women, when compared to Caucasian women.[6,7] Rather, it is proposed that since there is a strong correlation between markers of type II diabetes and obesity within African women, that the possible mechanism of cardiovascular risk could be attributed secondarily to the initial effect of obesity on the development of diabetes which, in turn, could lead to CVD.[7]

Furthermore, several studies have shown that acute or chronic stress may lead to dysregulation of the hypothalamic pituitary adrenal (HPA) axis which in turn is mediated by certain inflammatory markers such as CRP and other stress hormones which include cortisol.[8-11] Free fatty acids which are released from increased visceral or abdominal adipose tissues also play a role in heightening levels of CRP.[11-14] Sequentially, heightened levels of cortisol are also linked to increased adiposity and subsequently increased inflammation, which consequently leads to increased cardiovascular disease risk.[10,11] Moreover, this linkage is further supported by the decrease in inflammatory mediators in individuals where weight loss was demonstrated.[15]

It is important to note however that although several of the above-mentioned studies have explored this relationship in detail, and that while guidelines have been set for other ethnic groups, [15] the investigation of African and Caucasian women from South Africa has been limited. It is thus imperative that these relationships be explored further within these ethnic groups within this country in order to fully substantiate and assess possible risk for CVD within these groups. This in turn, may subsequently assist researchers to isolate possible mechanisms of action of hypertension within these groups and accordingly allow for better clinical approaches to this disease which

may consequently reduce its potential to influence future cardiovascular health negatively.

Materials and Methods

Study design

The POWIRS (Profiles of Obese Women with Insulin Resistance Syndrome) study consisted of two cross-sectional studies, of which the first was performed on 102 apparently healthy urban African women. This study was then repeated on a group of 115 Caucasian women. The two groups were matched on the basis of age and BMI. Attempts were made to choose African women of a higher socio-economic status, and thus these women were recruited from the employees of a government institution. The dietician within this institution subsequently recruited these subjects while taking the initial study design into account. The Caucasian women were recruited from an urban area with the assistance of a research nurse who advertised for and matched participants. All participants lived in the Potchefstroom district, South Africa.

There were two groups chosen based on the BMI guidelines of the Report of a World Health Organization Consultation on Obesity [16] namely:

(i) normal range BMI: 18.5 – 24.9 kg/m^2 (BMI $\leq$ 25 kg/m^2);

(ii) overweight and obese range: BMI $\geq$ 25 kg/m^2.

Exclusion criteria were: pregnancy, lactation, diabetes mellitus or an oral temperature above 37°C. Comprehensive results of CRP readings were only available for 101 African subjects.

Following the introduction of the subjects to the set-up and the explanation of experimental procedures that were being used, the subjects each signed an informed consent form. The Ethics Committee of the North-West University approved the study, and all procedures followed were in accordance with institutional guidelines. A participant sheet that guided each subject through the different research stations

was received by each subject and was subsequently signed at each station. All anthropometric measurements (except height and weight measurements) were taken during the course of the evening after which all participants received an identical supper that excluded alcohol or caffeine at 20:00. All subjects were asleep before 23:00 and fasted overnight. At 6:00 the following morning urine samples were collected, which were subsequently followed by weight, height and blood pressure measurements. A fasting blood sample was taken, after which a two-hour glucose tolerance test commenced. All subjects then received breakfast and personal information sheets regarding their own blood pressure, blood glucose, haemoglobin, etc. indicating where further testing and/or treatment was necessary. All the subjects were given a small financial compensation and were transported back to their destinations.

All data was collected from the African subjects during March-April 2003, and the samples were assayed during August 2003. The Caucasian subject data collection was done during August 2004 and the assays were completed during October 2004.

Anthropometric measurements

Height (stature), weight, seven skinfold thicknesses, abdominal sagittal diameter and four body circumferences of the subjects were measured by a qualified anthropometrist with calibrated instruments using standard methods (Precision Health Scale, A & D Company, Japan; Invicta Stadiometer, IP 1465, UK; Holtain unstretchable metal tape; John Bull callipers). Prediction equations based on the skinfolds were used to determine the fat percentages of the lean and overweight subjects whereas the fat percentage in obese subjects was determined using the body circumferences. All measurements were standardised and taken in triplicate.

Cardiovascular measurements

A 7-minute resting continuous measurement of cardiovascular parameters was taken whilst in the supine position using the Finometer device. (FMS, Finapres Measurement Systems, Arnhem, Netherlands) All cardiovascular variables were calculated by the Finometer and the data was then stored on a hard disk. Using the Beatscope 1.1 software programme each subject's heart rate (HR), systolic (SBP) and diastolic blood pressure (DBP), stroke volume, cardiac output, total peripheral resistance and arterial compliance were determined by integrating the subject's age, gender, body mass and height. Duplicate blood pressure readings were taken using a single-headed stethoscope and a mercury sphygmomanometer (Model ALPK2) both before and after the Finometer measurements. The first and fifth Korotkoff Phases were recorded as the SBP and DBP, respectively.

Biochemical analyses

High-sensitivity C-reactive protein (hs-CRP) levels were determined using blood serum samples that were analysed with a High-Sensitivity C-Reactive Protein Kit from Immage® Immunochemistry Systems. (Cat No. 474630, Beckham Coulter, Inc.). Serum cortisol was measured with $_{125}$I RIA Coat-a-count kit (Diagnostic Products Corporation, Cat. No. TKC01).

Statistical analyses

Data was analysed using Microsoft Excel for all mathematical calculations and graphing and further statistically analysed by means of the software computer package STATISTICA 6.0. All data was corrected for age, smoking and alcohol

consumption. Independent *t*-tests were performed to compare the two ethnic groups in terms of age, anthropometric, cardiovascular and biochemical variables. Partial correlations were performed to determine the correlations of both CRP and cortisol with various anthropometric and cardiovascular variables, while adjusting for age, smoking and alcohol consumption. Pearson's correlations were used to determine whether the correlations of the African and Caucasian women differed significantly ($P<0.05$). Means and standard deviations were determined using ANCOVA. The subjects of each ethnic group were divided into normotensive (NT) and hypertensive (HT) groups according to WHO recommendations for the determination of hypertensiveness[17]. Significant differences within each ethnic group were determined between each of the groups (NT and HT) by ANCOVA analyses, for both cardiovascular and anthropometric variables, while adjusting for age, smoking and alcohol consumption. Regression analyses were used to examine the relationship between CRP, cortisol and other parameters. Logistic regression using multiple regression analyses was used within each ethnic group to determine the relationship between cardiovascular and anthropometric variables and hypertension whilst using hypertensive BP as a dichotomous variable.

Results

Table 1 represents the overall means of both cardiovascular and anthropometric variables between African and Caucasian women. Waist circumference, WC, is the only anthropometric variable that exhibited significantly higher values within Caucasians when compared to African values. Conversely, all cardiovascular variables (Systolic blood pressure, SBP; Diastolic blood pressure, DBP; Cardiac Output, CO; Stroke Volume, SV; Total peripheral resistance, TPR; Windkessel Compliance, Cw) demonstrated significant differences ($p \leq 0.05$) between African and Caucasian mean values, with CO and SV displaying significantly elevated values within Caucasians. Caucasian women exhibited a significantly elevated cortisol when comparing different ethnic values whereas both African and Caucasian women demonstrated heightened CRP levels. African women exhibited a significantly higher peripheral vascular response as compared to Caucasian women when comparing vascular values.

Table 2 represents the overall mean characteristics of both the normotensive/hypertensive Caucasian and African women subjects after adjusting for age, smoking and alcohol usage. There was an overall trend within both African and Caucasian women in the anthropometric measurements (i.e. WC; body:mass index, BMI) of a significant increase in mean values once the hypertensive range was approached.

The resting central cardiac pattern (SV and CO) (Figure 2) has a propensity to significantly increase ($p \leq 0.05$) between normotensive and hypertensive values within Caucasian women. Conversely, this trend did not occur within African women. The central cardiac values, CO and SV exhibited a significant difference between the

African and Caucasian values in both the normotensive and hypertensive ($p = 0.00$) ranges (results not shown). Total peripheral resistance (TPR) (Figure 1) displays a trend of increase within both African and Caucasian women but this increase is only significant ($p \leq 0.05$) within African women. Windkessel compliance (Cw) has a tendency to decrease within both African and Caucasian women when approaching the hypertensive ranges yet, as in TPR, this tendency is only significant within African women (Figure 1). Both systolic (SBP) and diastolic blood pressures (DBP) increase significantly ($p \leq 0.05$) with increasing blood pressure (BP) within both African and Caucasian women. Nonetheless this inclination was not significant for SBP when trending towards the BMI ≥ 25 range within Caucasian women (results not shown). Finally, both cortisol and C-reactive protein (CRP) exhibited an increase in their values within both African and Caucasian women with increasing BP. However, these differences were not significant. Cortisol did however exhibit significant differences between Caucasian and African values for the normotensive ranges (results not shown).

Table 3 and Table 4 exhibit the correlations (adjusted for age, smoking and alcohol usage) between lnCRP and lnCortisol with various cardiovascular and anthropometric values of both African and Caucasian women within certain ranges. In Table 3, African women exhibited highly significant correlations between lnCRP and both WC and BMI within the hypertensive ranges whereas this result was not shown in Caucasian women. Both Caucasian and African women exhibited significant correlations between lnCRP, lnCortisol and both WC and BMI within the normotensive range. (Table 3 and Table 4)

Caucasian women showed significant correlations of lnCRP with both SV ($r = 0.39$; $p = 0.03$) and Cw ($r = 0.49$; $p = 0.00$) in the BMI ≥ 25 range (results not shown) and

the hypertensive range [(SV: r = 0.84; p= 0.01); (Cw: r = 0.83; p = 0.01)] (Table 3). Conversely, African women showed highly significant correlations of lnCRP with all cardiovascular variables (excluding DBP) in the hypertensive range (Table 3) and significant correlations with only TPR (r = 0.43; p = 0.02), Cw (r = 0.59; p = 0.00), SBP (r = 0.57; p = 0.00) and DBP (r = 0.44; p = 0.01) within the BMI $\geq$ 25 range (results not shown).

The hypertensive (HT) range within Caucasian women showed a significant correlation of lnCortisol with both Cw (r = 0.81; p = 0.02) and DBP (r = 0.80; p= 0.03) (Table 4). On the other hand, Africans only exhibited correlations of lnCortisol with TPR (r = 0.66; p = 0.048), Cw (r = 0.82; p = 0.00) and SBP (r = 0.73; p = 0.01) within the hypertensive range (Table 4).

Logistic regression of all variables against hypertension showed that the cardiac and vascular values (SBP, DBP, SV, Cw) show a significant contribution towards hypertension within African women. However, this was not the case within Caucasian women where SBP and DBP seem to play a far more significant role in hypertension within this ethnic group (Table 5).

TABLE 1: Descriptive statistics of cardiovascular and anthropometric variables between African and Caucasian Women.

	AFRICAN WOMEN MEAN (CI±95%) N = 102	CAUCASIAN WOMEN MEAN (CI±95%) N = 115
Age	31.12(29.43;32.81)	30.98(29.17;32.79)
WC (cm)	**81.62(79.02; 84.22)[a]**	**85.98(83.18;88.76)[a]**
BMI (kg/m^2)	27.98(26.74;29.23)	28.51(27.16;29.85)
SBP (mmHg)	**129.82(125.97;133.66)[b]**	**125.24(123.03;127.45)[b]**
DBP (mmHg)	**77.68(75.58; 79.78)[c]**	**72.30 (70.61;73.99)[c]**
CO (L/min)	**5.72(5.49;5.95)[d]**	**7.09(6.73;7.45)[d]**
SV (mL)	**84.67(81.84;87.51)[e]**	**97.94(93.14;102.74)[e]**
TPR (mmHg.s/mL)	**1.10(1.04;1.15)[f]**	**0.84(0.80;0.88)[f]**
Cw (mL/mmHg)	**1.85(1.79;1.91)[g]**	**2.29(2.21;2.36)[g]**
hsCRP (mg/L)	5.54(3.19;7.88)	3.29(2.57;4.01)
Cortisol(nmol/mL)	**455.74(402.37;509.11)[h]**	**604.90(545.23;664.56)[h]**

CI, Confidence Interval; WC, waist circumference; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure, CO, cardiac output; SV, stroke volume; TPR, total peripheral resistance; Cw, Windkessel compliance, ;hsCRP, High-sensitivity C-reactive protein. All data were adjusted for age, smoking and alcohol consumption and tested at a 95% confidence interval. Significant differences ($p \leq 0.05$) between variables in the groups are indicated with the same superscript letters.

TABLE 2: Cardiovascular and anthropometric variables between Normotensive and Hypertensive African and Caucasian Women.

	AFRICAN WOMEN		CAUCASIAN WOMEN	
	Normotensive (NT) N = 81	**Hypertensive (HT) N = 21**	**Normotensive (NT) N = 100**	**Hypertensive (HT) N = 12**
Age	**30.02(28.28; 31.77)[a]**	**36(31.49; 40.51)[a]**	30.76(28.94; 32.59)	35.58(29.15; 42.02)
BMI (kg/m^2)	**27.31(25.96; 28.65)[b]**	**30.59(27.51; 33.67)[b]**	27.94(26.54; 29.34)	31.79(26.54; 37.05)
WC (cm)	**80.01(77.17; 82.86)[c]**	**87.81(81.83; 93.79)[c]**	**84.54(81.65; 87.42)[h]**	**94.60(84.37; 104.83)[h]**
SBP (mmHg)	**122.93(120.90; 124.96)[d]**	**156.37(144.58; 168.15)[d]**	**122.05(120.43; 123.67)[i]**	**146.91(138.81; 155.00)[i]**
DBP (mmHg)	**74.34(72.50; 76.18)[e]**	**90.58(86.53; 94.64)[e]**	**70.89(69.27; 72.51)[j]**	**82.12(75.12; 89.13)[j]**
hsCRP (mg/L)	5.55(2.64; 8.45)	5.50(3.04; 7.95)	3.27(2.45; 4.10)	3.50(2.05; 4.95)
Cortisol (nmol/mL)	462.24(397.84; 526.63)	430.68(348.22; 513.15)	610.29(545.70; 674.88)	534.87(318.67; 751.07)

CI, Confidence Interval; hsCRP, High-sensitivity C-reactive protein; WC, waist circumference; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. All data were adjusted for age, smoking and alcohol consumption and tested at a 95% confidence interval. Significant differences ($p \leq 0.05$) between variables in the groups are indicated with the same superscript letters.

TABLE 3: Correlations of Cardiovascular and anthropometric variables with lnCRP in normotensive and hypertensive African and Caucasian women.

	AFRICAN WOMEN				CAUCASIAN WOMEN			
	Normotensive		Hypertensive		Normotensive		Hypertensive	
	r	p	r	p	r	p	r	p
lnCortisol (nmol/mL)	0.19	0.59	0.29	0.82	0.21	0.50	0.31	0.89
WC (cm)	0.46	**0.00**	0.69	**0.03**	0.39	**0.01**	0.53	0.45
BMI (kg/m^2)	0.37	**0.02**	0.80	**0.00**	0.43	**0.00**	0.52	0.48
CO (L/min)	0.28	0.18	0.66	**0.04**	0.29	0.15	0.52	0.47
SV (mL)	0.19	0.56	0.73	**0.01**	0.21	0.47	0.84	**0.01**
TPR (mmHg.s/mL)	0.28	0.17	0.74	**0.01**	0.31	0.09	0.59	0.33
Cw (mL/mmHg)	0.24	0.34	0.85	**0.00**	0.18	0.63	0.83	**0.01**
SBP (mmHg)	0.31	0.10	0.79	**0.00**	0.81	0.97	0.53	0.47
DBP (mmHg)	0.16	0.73	0.64	0.06	0.14	0.84	0.62	0.26

CRP, C-reactive protein; WC, waist circumference; BMI, body mass index; CO, cardiac output; SV, stroke volume; TPR, total peripheral resistance; Cw, Windkessel compliance; SBP, systolic blood pressure; DBP, diastolic blood pressure; SD, standard deviation. All data were adjusted for age, smoking and alcohol consumption and tested at a 95% confidence interval. Significant differences ($p<0.05$) are highlighted in **bold**. Significant differences ($p \leq 0.05$) between variables in the groups are indicated with the same superscript letters.

TABLE 4: Correlations of Cardiovascular and anthropometric variables with lnCortisol in normotensive and hypertensive African and Caucasian women.

	AFRICAN WOMEN				CAUCASIAN WOMEN			
	Normotensive		Hypertensive		Normotensive		Hypertensive	
	r	p	r	p	r	p	r	p
lnCRP (mg/L)	0.17	0.68	0.18	0.97	0.17	0.68	0.15	0.99
WC (cm)	0.46	**0.00**	0.23	0.93	0.38	**0.02**	0.53	0.46
BMI (kg/m^2)	0.35	**0.04**	0.28	0.84	0.41	**0.00**	0.52	0.48
CO (L/min)	0.28	0.17	0.37	0.65	0.30	0.13	0.51	0.51
SV (mL)	0.14	0.83	0.36	0.68	0.16	0.72	0.70	0.11
TPR (mmHg.s/mL)	0.28	0.18	0.66	**0.048**	0.32	0.09	0.59	0.32
Cw (mL/mmHg)	0.23	0.36	0.82	**0.00**	0.18	0.66	0.81	**0.02**
SBP (mmHg)	0.24	0.36	0.73	**0.01**	0.16	0.72	0.64	0.22
DBP (mmHg)	0.14	0.84	0.62	0.09	0.18	0.65	0.80	**0.03**

CRP, C-reactive protein; WC, waist circumference; BMI, body mass index; CO, cardiac output; SV, stroke volume; TPR, total peripheral resistance; Cw, Windkessel compliance; SBP, systolic blood pressure; DBP, diastolic blood pressure; SD, standard deviation. All data were adjusted for age, smoking and alcohol consumption and tested at a 95% confidence interval. Significant differences ($p<0.05$) are highlighted in **bold**. Significant differences ($p \leq 0.05$) between variables in the groups are indicated with the same superscript letters.

TABLE 5: Independent associations of cardiovascular and anthropometric variables with hypertension.

Caucasian women				African women			
Independent variables	**Partial R^2**	**Beta**	**P-value**	**Independent variables**	**Partial R^2**	**Beta**	**P-value**
AGE	0.77	-0.08	0.48	AGE	0.89	-0.03	0.46
BMI	0.93	-0.03	0.87	BMI	0.96	-0.05	0.42
WC	0.95	0.11	0.63	WC	0.96	0.04	0.51
SBP	0.81	0.69	0.00	SBP	0.99	1.58	0.00
DBP	0.96	-1.13	0.00	DBP	0.99	-0.85	0.00
MAP	0.97	0.00	0.99	MAP	1.00	-0.31	0.12
SV	0.84	0.59	0.39	SV	0.94	0.15	0.02
CO	0.93	-0.71	0.49	CO	0.97	0.09	0.28
TPR	0.95	-1.30	0.33	TPR	0.97	0.08	0.32
Cw	0.79	-0.96	0.14	Cw	0.97	-0.18	0.03
CRP	0.88	-0.49	0.53	CRP	0.87	-0.04	0.27
lnCRP	0.88	0.50	0.53	lnCRP	0.85	0.03	0.31
Cortisol	0.93	0.17	0.87	Cortisol	0.99	0.05	0.59
lnCortisol	0.92	-0.25	0.79	lnCortisol	0.99	-0.02	0.84

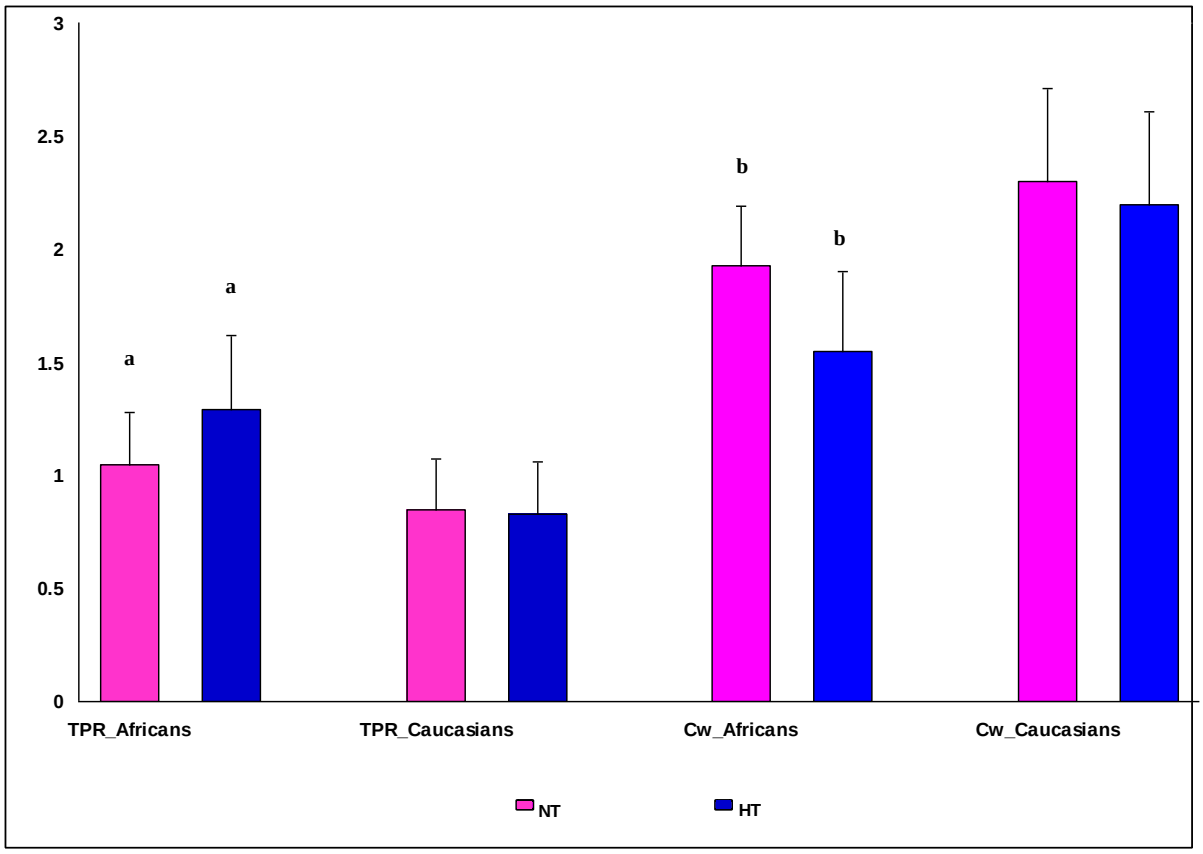

FIGURE 1: Resting Mean (±SD) Vascular Responses of African and Caucasian Women between Normotensive (BP ≤ 140/90mmHg) and Hypertensive (BP ≥ 140/90mmHg) groups. SD, Standard deviation; BP, Blood Pressure; TPR, Total Peripheral Resistance (mmHg.s/mL); Cw, Windkessel Compliance (mL/mmHg); NT, normotensive; HT, hypertensive.Values are adjusted for age, smoking and alcohol consumption and tested at a 95% confidence interval. Bars with the same superscript letter differ significantly.

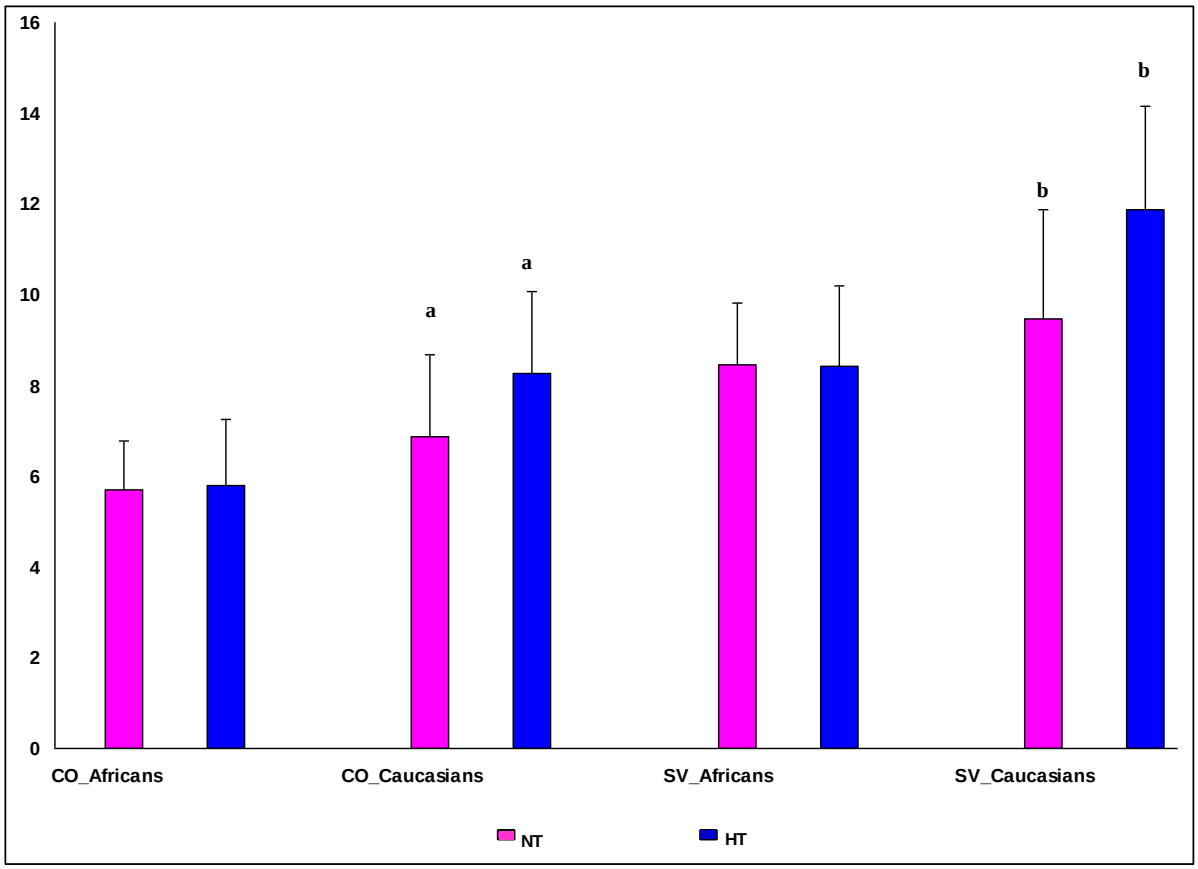

FIGURE 2: Resting Mean (±SD) Cardiac Responses of African and Caucasian Women between Normotensive (BP ≤ 140/90mmHg) and Hypertensive (BP ≥ 140/90mmHg) groups. SD, Standard deviation; BP, Blood Pressure; CO, Cardiac Output (L/min); SV, Stroke Volume (L); Cw, Windkessel Compliance (mL/mmHg); NT, normotensive; HT, hypertensive. Values are corrected for age, smoking and alcohol consumption and tested at a 95% confidence interval. Bars with the same superscript letter differ significantly.

Discussion

The aim of this study was to investigate the contribution of CRP, cortisol and hypertension to the increased likelihood of cardiovascular disease in both African and Caucasian women from South Africa. This study is relevant in that it describes relationships between various cardiovascular risk markers and hypertension within ethnic groups that, insofar as the author has ascertained, have been neglected.

Caucasian women exhibited an increased central cardiac activity when the hypertensive range was approached as demonstrated by the increased CO within this range (Table 2 and Figure 2). There is also a significant difference between normotensive and hypertensive SBP and DBP values respectively for Caucasian women (Table 2). These observations, in conjunction with the decreased cortisol levels within the hypertensive group, suggest increased sympathetic reactivity with an accompanying hypocortisolism. This corresponds to more recent studies[4, 10, 18] where it was found that hypocortisolism has in some cases been related to hypertension.

African women, conversely, showed increased vascular reactivity when the hypertensive range was approached (Table 2 and Figure 1). Cw significantly decreased within the hypertensive range within this group with a corresponding significant increase in TPR. Hypocortisolism was exhibited within the hypertensive African women (Table 2). This once again possibly suggests an over-stimulated HPA axis leading to hypertension in association with hypocortisolism.[4, 10, 18] Urbanisation has been previously shown as a psychosocial stressor that contributes to increased cardiovascular reactivity and hence, hypertensiveness.[2-5] Subsequently, since the African women within this study are urbanised, one could hypothesise that this could be a possible explanation for the above-mentioned results. The increased

cardiovascular reactivity within hypertensive ranges in this ethnic group corresponds to previous studies that have found an increased relative risk for hypertension in both Africans and African-Americans when compared to other ethnic groups due to increased cardiovascular reactivity measurements when a specific stressor was applied.[2, 3, 5, 19-23]

CRP has been significantly correlated to WC and BMI within the hypertensive ranges within African women. However, this observation does not correspond to Caucasian women (Table3). These observations correspond to previous studies, in which it was shown that CRP had its strongest correlations with BMI and WC, [6, 24-26] and how these correlations are most noticeable amongst African-American women and African women.[6, 27] BMI and WC means within African women also significantly increased when the hypertensive range was approached. (Table 2). This increase in mean body fat indicators could possibly explain the high correlations with CRP when one considers the relationship between IL-6 and CRP concentrations. IL-6 is expressed within adipose tissues and this inflammatory marker in turn stimulates the production of CRP in the liver.[24, 28] In addition, IL-6 has been shown to increase with increasing adiposity in healthy men and women, with higher amounts being expressed within visceral fat depots when compared to subcutaneous fat depots.[12, 13, 29] This mechanism subsequently could plausibly explain the observed higher correlation with WC as compared to BMI in African women, as BMI represents overall adiposity whereas WC represents abdominal/visceral adiposity. On the contrary, anthropometric variables did not correlate significantly with cortisol when hypertensive ranges were approached in both African and Caucasian women (Table 4) but there was a significant correlation between these variables within the normotensive ranges within both race groups. One could postulate that this could

possibly be attributed to the observation that both Caucasian and African women already exhibited elevated means of both BMI and WC within the normotensive ranges (Table 2) before the hypertensive range was approached and subsequently these individuals could have already been exhibiting HPA axis reactivity [9-11, 29, 30, 31] to the heightened adiposity and thus a significant correlation to both CRP and cortisol within this range. Since the duration of obesity is unknown, the loss of these correlations within the hypertensive ranges could possibly be attributed to HPA axis dysregulation [4, 10, 18] which is further supported by the observed hypocortisolism within this range (Table 2).

CRP values were shown to be significantly positively associated with hypertensive SBP in African women. CRP also demonstrated significant correlations with all cardiovascular variables within the hypertensive range in African women. However, the highest correlations were exhibited with the peripheral reactivity indicators, TPR ($r=0.74$; $p=0.01$) and Cw ($r=0.85$; $p=0.00$), (Table 3). Once again, as mentioned previously these observations in conjunction with the significant differences between the mean values of these indices (Table 2), suggest a complementary peripheral cardiovascular reactivity accompanying an increase in blood pressure within African women. This in conjunction with the significant associations with CRP within this group is congruous with previous studies where CRP was reported to be associated with hypertensive BP values with greater associations occurring among women and black ethnic groups.[28, 29, 33-42] In addition, increases in blood pressure within African-American women have been previously demonstrated with a simultaneous occurrence of peripheral vasodilation and a greater indexed peripheral resistance (as compared to Caucasians).[21, 43] Moreover, CRP levels have previously corresponded to an increased likelihood of peripheral arterial disease amongst United States adults

independent of smoking, waist circumference, body mass index, hypertension and other confounders.[44] Thus among African women it can be deduced that the high correlations between CRP and the peripheral cardiovascular variables, TPR and Cw, suggest a greater risk of the development of hypertension and consequently the development of possible cardiovascular disease. In addition to these observations the overall mean values of CRP was higher within Africans when compared with Caucasians (Table 1), however both ethnic groups demonstrated CRP levels that fall within the high risk group for CVD (values exceeding 3.0mg/L).[45] Caucasian women displayed a significant correlation between CRP and both SV (r=0.84; p=0.01) and Cw (r=0.83; p=0.01), (Table 3). Previous studies have found that increased CRP levels have been associated with decreased nitric oxide production, [37, 45] which subsequently leads to the destruction of endothelial functions such as vasodilation. Inhibition of nitric oxide in turn leads to increased α-adrenergic effects which include increased vasoconstriction and sympathetic activity.[46] Following this, one could conceivably suggest that the inhibition of nitric oxide could possibly explain the decrease in Cw and the significant increases in CO and SV (Table 2) in conjunction with the high correlations of CRP with SV and Cw (Table 3). Thus the increased CRP in Caucasian women could feasibly cause endothelial dysfunction which in turn could lead to hypertension and subsequent cardiovascular dysfunction. Consequently both Caucasian and African women exhibit possible risk markers for future cardiovascular disease.

Cortisol correlated significantly with both Cw (r=0.82; p=0.00) and TPR (r=0.66; p=0.048) in African women whereas in Caucasian women there was only a significant correlation with Cw (r=0.81; p=0.02) (Table 4). Decreased cortisol levels were also exhibited during the hypertensive range within both race groups (Table 2) which

could possibly be indicative of the hypocortisolism that has been previously displayed during hypertension, especially within African women.[4, 10, 18] Suggestions have been made that this could possibly be attributed to dysregulation of the HPA axis which in turn is mediated by certain inflammatory markers such as CRP.[15] Thus the high correlation between Cw and TPR within these ethnic groups could still be attributed to the initial effects of increased cortisol production by the HPA axis in response to stress. This in turn could also further support the significant increase in the CO and SV means when the hypertensive range was approached in Caucasian women (Table 2) and the significant increase in TPR and Cw means in African women in the hypertensive range (Table2). A possible mechanism could be that of the effect of norepinephrine release by cortisol stimulation and the subsequent α-adrenergic response.[46]

African women illustrated significant correlations with SBP, DBP, SV and Cw against hypertension during multiple regression analyses (Table 5). Nevertheless, the highest correlations were yet again with Cw and SV within this group which once again suggests the possibility of the dysregulation of the HPA axis in conjunction with norepinephrine release by cortisol stimulation within this group.[4, 10, 15, 18, 46] Thus among African women a suggestion of HPA axis dysregulation is most likely. However, among Caucasian women these observations were not reiterated. On the contrary it was found that SBP and DBP seemed to play a far more significant role in hypertension within this group. This once again supports the previous observations where the initial increase in cortisol release followed by HPA axis dysregulation could possibly increase the SBP and DBP values due to increase in norepinephrine and epinephrine release which subsequently could lead to hypertension. [4, 10, 15, 18, 46]

In conclusion, both African and Caucasian women displayed possible HPA axis dysregulation, which in turn could plausibly explain the hypertension within these groups. The roles of CRP and cortisol still remain rather uncertain within these groups. However, in Caucasian women, cortisol presented a higher likelihood of contribution towards hypertension than in African women, whereas within African women CRP seemed to play a larger role (Table 3 and Table 4).

A possible limitation of this study could be the duration of obesity amongst the participants which could possibly influence the habituation/adaptation of physiological resources. Furthermore one needs to consider that hsCRP is a non-specific marker for inflammation and thus although the participants within this study were apparently healthy, the higher hsCRP levels could possibly be explained by other unknown inflammatory conditions.[45] Additionally, the time difference in data collection could have possibly influenced the results, although temperatures were similar for both collection periods (autumn and spring). The significance of this study could have also been further substantiated if more data were obtained regarding the use of hormone replacement therapy (HRT) or oral contraceptives (OC) as these could possibly affect the CRP values within this study.

Future studies should include further examination of the role of HPA axis dysregulation within these groups and the possible mechanisms of action regarding the incidence of hypertension considering these effects.

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REFERENCES

(1) Opie LH, Seedat YK. Hypertension in sub-Saharan African populations. *Circulation* 2005; **112**: 3562-3568.

(2) Gerin W, Pickering TG, Glynn L, Christenfeld N, Schwartz A, Carroll D *et al*. An historical context for behavioral models of hypertension. *J Psychosom Res* 2000; **48**: 369-377.

(3) Hinderliter AL, Blumenthal JA, Waugh R, Chilukuri M, Sherwood A. Ethnic differences in left ventricular structure: relations to hemodynamics and diurnal blood pressure variation. *AJH* 2004; **17**: 43-49.

(4) Huisman HW, van Rooyen JM, Malan NT, Eloff FC, Malan L, Laubscher PJ *et al*. Prolactin, testosterone and cortisol as possible markers of changes in cardiovascular function associated with urbanization. *J Hum Hypertens* 2002; **16**: 829-835.

(5) Seedat YK. The prevalence of hypertension and the status of cardiovascular health in South Africa. *Ethn Dis* 1998; **8**: 394-397.

(6) Schutte AE, van Vuuren D, van Rooyen JM, Huisman HW, Schutte R, Malan L *et al*. Inflammation, obesity and cardiovascular function in African and Caucasian women from South Africa: the POWIRS study. *J Hum Hypertens* 2006; **20**: 850-859.

(7) Schutte AE, Huisman HW, van Rooyen JM, Schutte R, Malan L, Reimann M *et al*. Should obesity be blamed for the high prevalence rates of hypertension in black South African women? *J Hum Hypertens* 2008; **22**: 528-536.

(8) Björntorp P, Rosmond R. Obesity and cortisol. *Nutrition* 2000; **16**(10): 924-936.

(9) Björntorp P. Do stress reactions cause abdominal obesity and comorbidities? *Obes Rev* 2001; **2**: 73-86.

(10) Kopp MS, Réthelyi J. Where psychology meets physiology: chromic stress and premature mortality - the Central-Eastern European health paradox. *Brain Res Bull* 2004; **62**: 351-367.

(11) Black PH. The inflammatory response is an integral part of the stress response: Implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X. *Brain Behav Immun* 2003; **17**: 350-364.

(12) Misra A, Vikram NK. Clinical and pathophysiological consequences of abdominal adiposity and abdominal adipose tissue depots. *Nutrition* 2003; **19**: 457-466.

(13) Frühbeck G, Salvador J. Role of adipocytokines in metabolism and disease. *Nutrition Research* 2004; **24**: 803-826.

(14) Ahima RS, Flier JS. Adipose tissue as an endocrine organ. *TEM* 2000; **11**(8): 327-332.

(15) Black PH. The inflammatory consequences of psychologic stress: Relationship to insulin resistance, obesity, atherosclerosis and diabetes mellitus, type II. *Med Hypotheses* 2006; **67**: 879-891.

(16) World Health Organization. Obesity: Preventing and managing the global epidemic. Report of a WHO consultation on obesity, June 3-5, 1997, Geneva, Switzerland. World Health Organisation: Geneva, 1997.

(17) WHO (World Health Organization). World Health Organization/ International Society of Hypertension (ISH) statement on management of hypertension. *J Hypertens* 2003; **21**: 1983-1992.

(18) Heim C, Ehlert U, Hellhammer DH. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychneuroendocrinolgy* 2000; **25**: 1-35.

(19) Malan NT, Van der Merwe JS, Huisman HW, Kruger A, Eloff FC, Pretorius PJ *et al*. A comparison of cardiovascular reactivity of rural blacks, urban blacks and whites. *Stress Medicine* 1992; **8**: 1-6.

(20) Gerin W, Pickering TG, Glynn L, Christenfeld N, Schwartz A, Carroll D *et al*. An historical context for behavioral models of hypertension. *J Psychosom Res* 2000; **48**: 369-377.

(21) Suarez EC, Saab PG, Llabre MM, Kuhn CM, Zimmerman E. Ethnicity, gender, and age effects on adrenoreceptors and physiological responses to emotional stress. *Psychophysiology* 2004; **41**: 450-460.

(22) Knox SS, Hausdorff J, Markovitz JH. Reactivity as a predictor of subsequent blood pressure. Racial differences in the coronary artery risk development in young adults (CARDIA) study. *Hypertension* 2002; **40**: 914.

(23) Liao Y, Tucker P, Okoro CA, Giles WH, Mokdad AH, Harris VB. REACH 2010 Surveillance for health status in minority communities- United States, 2001-2002. *MMWR. Surveillance summaries: Morbidity and mortality weekly report. Surveillance summaries.* 2004; **53**(6): 1-36.

(24) Rexrode KM, Pradhan A, Manson JE, Buring JE, Ridker PM. Relationship of total and abdominal adiposity with CRP and IL-6 in women. *AEP* 2003; **13**(10): 674-682.

(25) Vikram NK, Misra A, Dwivedi M, Sharma R, Pandey RM, Luthra K *et al*. Correlations of C-reactive protein levels with anthropometric profile,

percentage of body fat and lipids in healthy adolescents and young adults in urban North India. *Atherosclerosis* 2003; **168**: 305-313.

(26) Lear SA, Chen MM, Birmingham CL, Frohlich JJ. The relationship between simple anthropometric indices and C-reactive protein: ethnic and gender differences. *Metabolism* 2003; **52**(12): 1542-1546.

(27) Albert MA, Glynn RJ, Buring J, Ridker PM. C-reactive protein levels among women of various ethnic groups living in the United States (from the Women's Health Study). *Am J Cardiol* 2004; **93**(10): 1238-1242.

(28) Libby P, Ridker PM. Inflammation and atherosclerosis: Role of C-reactive protein in risk assessment. *Am J Med* 2004; **116**(6A): 9S-16S.

(29) Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis* 2000; **148**: 209-214.

(30) al' Absi M, Arnett DK. Adrenocorticol responses to psychological stress and risk for hypertension. *Biomed & Pharmacother* 2000; **54**: 234-244.

(31) Whitworth JA, Brown MA, Kelly JJ, Williamson PM. Mechanisms of cortisol-induced hypertension in humans. *Steroids* 1995; **60**: 76-80.

(32) Malan NT, Brits JS, Eloff FC, Huisman HW, Kruger A, Laubscher PJ *et al*. The influence of acculturation on endocrine reactivity during acute stress in urban black males. *Stress Medicine* 1996; **12**: 55-63.

(33) Kullo IJ, McConnell JP, Bailey KR, Kardia SL, Bielak LF, Peyser PA *et al*. Relation of C-reactive protein and fibrinogen to coronary artery calcium in subjects with systemic hypertension. *Am J Cardiol* 2003; **92**: 56-58.

(34) Srikumar N, Hopkins PN, Jeunemaitre X, Hunt SC, Williams GH. Inflammatory marker C-reactive protein in hypertensive subjects. *AJH* 2002; **15**(4): 227A.

(35) Sesso HD, Buring JE, Rifai N, Blake GJ, Gaziano JM, Ridker RM. C-reactive protein and the risk of developing hypertension. *JAMA* 2003; **290**: 2945-2951.

(36) de Ferranti S, Rifai N. C-reactive protein and cardiovascular disease : a review of risk prediction and interventions. *Clin Chim Acta* 2002; **317**: 1-15.

(37) Sung KC, Suh JY, Kim BS, Kang JH, Kim H, Lee MH *et al*. High sensitivity C-reactive protein as an independent risk factor for essential hypertension. *AJH* 2003; **16**: 429-433.

(38) Schillaci G, Pirro M, Gemelli F, Pasqualini L, Vaudo G, Marchesi S *et al*. Systolic and pulse pressure as major culprits of increased plasma C-reactive protein levels in patients with newly diagnosed hypertension. *AJH* 2003; **16**(5): 258A.

(39) Saito M, Ishimitsu T, Minami J, Ono H, Ohrui M, Matsuoka H. Relations of plasma high-sensitivity C-reactive protein to traditional cardiovascular risk factors. *Atherosclerosis* 2003; **167**: 73-79.

(40) Williams MJA, Williams SM, Milne BJ, Hancox RJ, Poulton R. Association between C-reactive protein, metabolic cardiovascular risk factors, obesity and oral contraceptive use in young adults. *Int J Obes* 2004; **28**: 998-1003.

(41) de Maat MP, Kluft C. Determinants of C-reactive protein concentration in blood. *Ital Heart J* 2001; **2**(3): 189-195.

(42) Kraus VB, Stabler TV, Luta G, Renner JB, Dragomir AD, Jordan JM. Interpretation of serum C-reactive protein (CRP) levels for cardiovascular disease risk is complicated by race, pulmonary disease, body mass index, gender, osteoarthritis. *Osteoarthritis Cartilage* 2007; **15**: 966-971.

(43) Hinderliter AL, Blumenthal JA, Waugh R, Chilukuri M, Sherwood A. Ethnic differences in left ventricular structure: relations to hemodynamics and diurnal blood pressure variation. *AJH* 2004; **17**: 43-49.

(44) Shankar A, Li J, Nieto J, Klein BEK, Klein R. Association between C-reactive protein level and peripheral arterial disease among US adults without cardiovascular disease, diabetes, or hypertension. *Am Heart J* 2007; **154**: 495-501.

(45) Labarrere CA, Zaloga GP. C-reactive protein: From innocent bystander to pivotal mediator of atherosclerosis. *Am J Med* 2004; **117**(7): 499-507.

(46) Opie LH. The Heart: Physiology from cell to circulation, 3rd edn. Lippencott/Raven Publishers: Philadelphia, 1998, 19-25pp, 276pp, 288pp.

CHAPTER 3

GENERAL FINDINGS AND CONCLUSIONS

INTRODUCTION

This chapter will present the main findings that were reported in this dissertation. Results will be discussed, interpreted, explained and compared to the relevant literature study from Chapter 1. Conclusions will be drawn and recommendations regarding future research into the relationship between C-reactive protein (CRP), cortisol and hypertension within African and Caucasian women will be made.

SUMMARY OF MAIN FINDINGS

The significant findings of this article reported for this dissertation were the following:

The relationship between cortisol, C-Reactive Protein and hypertension in the development of cardiovascular dysfunction in African and Caucasian women: the POWIRS study (Chapter 2).

The aim of this study was to investigate the contribution of CRP, cortisol and hypertension to the increased likelihood of cardiovascular disease in both African and Caucasian women from South Africa. It was hypothesised that (a) African women,

compared to Caucasian women would present with higher CRP levels and subsequently, positive associations with hypertension and that (b) African women, compared to Caucasian women would present with lower cortisol levels and subsequently, positive associations with elevated vascular responses and consequently, hypertension. Results of African women showed that CRP levels were elevated as compared to Caucasian women and that CRP within African women significantly correlated with total peripheral resistance (TPR), Windkessel compliance (Cw) and systolic blood pressure (SBP) within the hypertensive range. Caucasian women also exhibited significant correlations of CRP with both stroke volume (SV) and Cw when the hypertensive range was approached. Subsequently, the first hypothesis was therefore accepted. Both African and Caucasian women exhibited hypocortisolism when the hypertensive range was observed. Furthermore, African women demonstrated lower resting cortisol levels as compared to Caucasian women. African women also presented with significant differences between vascular markers Cw and TPR when comparing these markers within the normotensive (NT) and hypertensive (HT) ranges, respectively. Caucasian women did not display these findings but on the contrary, exhibited significant differences between the cardiac markers SV and cardiac output (CO) when comparing these ranges. Moreover, cortisol displayed significant correlations with both Cw and TPR within African women in the HT range. Thus the second hypothesis was also accepted.

COMPARISON TO RELEVANT LITERATURE

Certain findings of this study are comparable to that found in previous literature. One such study is that of Schutte A.E. *et al*. (2006), where it was found that African

women showed significantly higher CRP and blood pressure (BP) readings when compared to their Caucasian counterparts. This was further supported by yet another study where African women exhibited elevated BP and higher HT rates in conjunction with higher arterial resistance and lower CO readings as compared to Caucasian women (Schutte *et al*., 2008). Hypocortisolism has previously been indicated within African women when hypertensiveness is shown (Huisman H.W. *et al.*, 2002).

Contradictory findings of this study were that the anthropometric variables body-mass index (BMI) and waist circumference (WC) did not correlate significantly with cortisol when the hypertensive range was approached (Björntorp P., 2001; Misra A. *et al*., 2003). Lowered cortisol levels were associated with hypertensiveness within both African and Causcasian women whereas previous studies have shown an associated hypercortisolism with hypertensiveness within these groups (al' Absi M. *et al.*, 2000; Kopp M.S. *et al.*, 2004).

CHANCE AND CONFOUNDING

Chance: Prior to discussing the main findings of this study one must consider several factors that may have caused weaknesses within the study and thus may have influenced the outcomes of the research.

The overall South African population is a diverse combination of several ethnic groups and cultures and subsequently the number of subjects within both ethnic group samples (Caucasian: 115; African: 102) may have been insufficient to identify etiological trends within the greater population as these samples were recruited from

the Potchefstroom district within the North-West Province which is not representative of the overall population of South Africa.

The possibility of chance must also be taken into account when one considers the results of this study. Statistics indicate that one out of twenty significant correlations may be because of chance if both forward stepwise regressions analyses and partial correlations were used.

Confounding factors: According to the inclusion criteria for this study the subjects were "apparently healthy" however the health status of the subjects is not a certainty. Other confounding factors such as HIV status, socio-economic status, diet and physical activity level may have influenced the results. Age, alcohol consumption and smoking, as possible confounders, were addressed by statistical adjustment.

WEAKNESSES OF THE STUDY

Weaknesses of the study included:

1. The duration of obesity was unknown which could possibly influence the habituation/adaptation of physiological resources.
2. hsCRP is a non-specific marker for inflammation and thus although the participants within this study were apparently healthy, the higher hsCRP levels could possibly be explained by other unknown inflammatory conditions.
3. Although temperatures were similar for both collection periods (autumn and spring), the time difference in data collection could have possibly influenced the results.

4. The significance of this study could have also been further substantiated if more data were obtained regarding the use of hormone replacement therapy (HRT) or oral contraceptives (OC) as these could possibly affect the hsCRP values within this study.

DISCUSSION OF MAIN FINDINGS

Both CRP and cortisol are associated with hypertension and subsequent cardiovascular disease, with higher associations occurring amongst African women when compared to their Caucasian women and African men counterparts (Schutte *et al*., 2006; Huisman *et al*., 2002). Africans have also shown a higher prevalence of hypertension when compared with Caucasians, Indians and those of mixed origin (Seedat *et al*., 1998; van Rooyen *et al*., 2000; van Rooyen *et al*., 2002). Urbanisation has been shown to play a significant role in increasing cardiovascular reactivity within Africans when compared to other groups (Seedat *et al*., 1998; van Rooyen *et al*., 2000; van Rooyen *et al*., 2002). Schutte A.E. *et al*. (2008) have demonstrated that African women have higher blood pressure and HT rates in conjunction with higher arterial resistance and lower CO as compared to their Caucasian counterparts. Moreover, hypocortisolism, in conjunction with urbanisation, has been proposed as a possible contributing factor to the increased prevalence of HT within African women (Huisman *et al*. 2002). However, the role of both CRP and cortisol in the observation of increased hypertension within both African and Caucasian groups in South Africa has not been fully examined.

This study endeavoured to explain the roles that CRP, cortisol and hypertension plays in the incidence of cardiovascular disease in both Africans and Caucasians within South Africa. Although the findings cannot be attributed to the overall African and Caucasian female population of South Africa, they can form the basis for possible future comprehensive studies.

Elevated CRP levels were observed within African women when compared to Caucasian women. Moreover, CRP within African women significantly correlated with total peripheral resistance (TPR), Windkessel compliance (Cw) and systolic blood pressure (SBP) within the hypertensive range. Significant correlations of CRP with both stroke volume (SV) and Cw were demonstrated among Caucasian women when the hypertensive range was approached. Both the above-mentioned observations within the Caucasian and African women samples could possibly be attributed to dysregulation of the hypothalamic-pituitary adrenal (HPA) axis which has been suggested in previous literature (Huisman *et al*., 2002; Kopp *et al*., 2004; Black, 2003; Heim *et al.*, 2000). Furthermore, the discernable hypocortisolism within both ethnic groups during the hypertensive range could possibly also be attributed to the dysregulation of the HPA axis (Huisman *et al*., 2002; Kopp *et al*., 2004; Black, 2003; Heim *et al.*, 2000). Additionally, African women presented with significant differences between vascular markers Cw and TPR when comparing these markers within the normotensive (NT) and hypertensive (HT) ranges, respectively. Conversely, Caucasian women did not display these findings but rather exhibited significant differences between the cardiac markers SV and cardiac output (CO) when comparing these ranges. Thus, these results suggest the possible diverse roles that

HPA axis dysregulation can play, in conjunction with the respective cardiac and vascular responses within both Caucasian and African women, on future cardiovascular risk for these groups.

CONCLUSION

In conclusion, both African and Caucasian women display possible HPA axis dysregulation, in conjunction with an observed increased vascular reactivity among African women and an increased central cardiac reactivity in Caucasian women, which in turn could plausibly explain the hypertension within these groups. The roles of CRP and cortisol still remain rather uncertain within these groups. However, within Caucasian women, cortisol presents a higher likelihood of contribution towards hypertension than within African women, whereas within African women CRP seems to play a larger role.

RECOMMENDATIONS

The following recommendations are proposed for future studies:

1. The mean age of the subjects within both of the sample groups of this study was relatively young and as such, prospective studies are recommended to further assess the possible long-term effects that both CRP and cortisol may have on cardiovascular dysfunction.

2. Since hsCRP readings could have been influenced by other unknown inflammatory conditions, it would be valuable to assess the influence (if any) that known inflammatory conditions may have on both physiological and cardiovascular risk markers.
3. The duration of obesity is yet another factor that needs to be taken into account. As the effects of both CRP and cortisol may differ depending on acute or chronic exposure, it would be constructive to consider the influence (if any) that the duration of obesity may have on these parameters.
4. Prospective studies should also include oral contraceptive and hormone replacement therapy use in the evaluation of CRP and its relationship to other cardiovascular risk factors due to their possible influence by elevating these readings (Williams, 2004).

REFERENCES

AL' ABSI, M. & ARNETT, D.K. 2000. Adrenocorticol responses to psychological stress and risk for hypertension. *Biomed & Pharmacother,* 54: 234-244.

BLACK, P.H. 2003. The inflammatory response is an integral part of the stress response: Implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X. *Brain Behav Immun,* 17: 350-364.

BJÖRNTORP, P. 2001. Do stress reactions cause abdominal obesity and comorbidities? *Obes Rev,* 2: 73-86.

HEIM, C., EHLERT, U. & HELLHAMMER, D.H. 2000. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychneuroendocrinolgy,* 25: 1-35.

HUISMAN, H.W., VAN ROOYEN, J.M., MALAN, N.T., ELOFF, F.C., MALAN, L., LAUBSCHER, P.J. & SCHUTTE, A.E. 2002. Prolactin, testosterone and cortisol as

possible markers of changes in cardiovascular function associated with urbanization. *J Hum Hypertens,* 16: 829-835.

KOPP, M.S. & RÉTHELYI, J. 2004. Where psychology meets physiology: chromic stress and premature mortality - the Central-Eastern European health paradox. *Brain Res Bull*, 62: 351-367.

MISRA, A. & VIKRAM, N.K. 2003. Clinical and pathophysiological consequences of abdominal adiposity and abdominal adipose tissue depots. *Nutrition,* 19: 457-466.

SCHUTTE, A.E., VAN VUUREN, D., VAN ROOYEN, J.M., HUISMAN, H.W., SCHUTTE, R., MALAN, L. & MALAN, N.T. 2006. Inflammation, obesity and cardiovascular function in African and Caucasian women from South Africa: the POWIRS study. *J Hum Hypertens*, 20: 850-859.

SCHUTTE, A.E., HUISMAN, H.W., VAN ROOYEN, J.M., SCHUTTE, R., MALAN, L., REIMANN, M., DE RIDDER, J.H., VAN DER MERWE, A., SCHWARZ, P.E.H. & MALAN, N.T. 2008. Should obesity be blamed for the high prevalence rates of hypertension in black South African women? *J Hum Hypertens*, 22: 528-536.

SEEDAT, Y.K. 1998. The prevalence of hypertension and the status of cardiovascular health in South Africa. *Ethn Dis,* 8: 394-397.

VAN ROOYEN, J.M., KRUGER, H.S., HUISMAN H.W., WISSING, M.P., MARGETTS, B.M., VENTER, C.S. & VORSTER, H.H. 2000. An epidemiological study of hypertension and its determinants in a population in transition: the THUSA study. *J Hum Hypertens*, 14: 779-787.

VAN ROOYEN, J.M., HUISMAN H.W., ELOFF, F.C., LAUBSCHER, P.J., MALAN, L., STEYN, S. & MALAN, N.T. 2002. Cardiovascular reactivity in black South African males of different age groups: the influence of urbanization. *Ethn Dis*, 12: 69-75.

WILLIAMS, M.J.A., WILLIAMS, S.M., MILNE, B.J., HANCOX, R.J. & POULTON, R. 2004. Association between C-reactive protein, metabolic cardiovascular risk factors, obesity and oral contraceptive use in young adults. *Int J Obes*, 28: 998-1003.