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THE CHARACTERISTICS OF CORONARY ARTERY DISEASE IN SOWETO

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand (Johannesburg) for the degree of Doctor of Philosophy

DECLARATION

I declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy to the University of Witwatersrand, Johannesburg. It has not previously been submitted for any degree or examination at this or any other university.

Lucas Mthetheli Ntyintyane

DEDICATION

RECESSIONAL

God of our fathers, known of old,
Lord of our far-flung battle-line,
Beneath whose awful Hand we hold
Dominion over palm and pine -
Lord God of Hosts, be with us yet,
Lest we forget - lest we forget!

- Rudyard Kipling

A PSALM OF LIFE

… Let us, then, be up and doing,
With a heart for any fate;
Still achieving, still pursuing,
Learn to labour and to wait.

- H.W Longfellow

Life is about effort, love and faith. I didn't walk this journey alone. God was my guide and my shield. He carried me through the storms and hurricanes of life. Today, a bright new day is dawning. I thank my parents, Sindile Elliot Ntyintyane and Nomonde Cordelia Ntyintyane, for all their love, sacrifices and support. Mama, Tata – I love you. You are my inspiration! Your life was not easy, but you gave us a home full of love and laughter. We lacked material possessions, but we lived a life of richness. You taught me to value work and to treat life as a special gift from God. I am glad you are here to witness this milestone in my life.

I thank my dear partner, Kululwa. You blessed me with your love; your footsteps gave me courage to walk the extra mile. Your prayers were my energy. Your life is a gift to me. Ndiyabulela. I love you.

To my brothers, Ayanda and Myolisi, I thank you. To my sister, Nomgcobo, the rose in the family, you are my lucky star.

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PRESENTATIONS AT CONFERENCES AND LECTURES

ORAL

1. Society for Endocrinology, Metabolism and Diabetes South Africa (SEMDSA) Congress in Durban, March 2004
   Ntyintyane, L., Raal, F.J., Paiker, J., Holden, N. and Ramela, N. *Postprandial Lipaemia in South African Blacks with established coronary artery disease.*
   Ntyintyane, L., Raal, F.J., Wing, J.R., Paiker, J., Holden, N. and Ramela, N. *Undiagnosed type 2 diabetes mellitus and insulin resistance are highly prevalent in South African Blacks with established coronary artery disease.*

2. Wits Health Science Research Day, August 2004
   Ntyintyane, L., Raal, F.J., Wing, J.R. and Paiker, J. et al. *Undiagnosed type 2 diabetes mellitus and insulin resistance are highly prevalent in South African Blacks with established coronary artery disease.*

3. South African Heart Association Congress, Cape Town, November 2006
   Ntyintyane, L., Stewart, S., Wilkinson, D. and Sliwa, K. *Incident and prevalent cases of heart disease presenting to a tertiary hospital in South Africa: initial results from the Heart of Soweto Study.*

4. Wits University Health Science Research Day, 2006
   Ntyintyane, L.M., Panz, V.R. and Raal, F.J. *Metabolic syndrome, undiagnosed diabetes mellitus and insulin resistance are highly prevalent in urbanized South African Blacks with coronary artery disease.*

5. SEMDSA and LASSA 2009 Congress, Sandton
   Ntyintyane L, Holland Z, Gill G, Raal F. *Carotid intima-media thickness is a predictor of coronary artery disease in South African black patients*.

Ntyintyane L, Panz V, Raal F, Gill G. *Postprandial hyperglycaemia in urbanized South African blacks with and without coronary artery disease*.

POSTER PRESENTATIONS

1. Biennial Congress of the South African Hypertension Society (SAHS), Cape Town, March 2005
   Ntyintyane, L., Raal, F.J. and Paiker, J. *Hypertension, if associated with features of the metabolic syndrome, appears to be atherogenic in South African Blacks*.

2. International Diabetes Federation (IDF), World Diabetes Congress, Cape Town, December 2006
   Ntyintyane, L., Raal, F.J., Panz, V.R. and Gill, G. *Prevalence of the metabolic syndrome using the National Cholesterol Education Program and International Diabetes Federation definitions in urbanized South African Blacks with established coronary artery disease.*

3. South African Heart Association Congress, Sun City, November 2007
   Ntyintyane, L., Stewart, S., Hansen, C., Wilkinson, D., Tibazarwa, K., Raal, F. and Sliwa, K. *The impact of urbanization on black patients presenting with coronary artery disease at Baragwanath Hospital, Cardiac Clinic, Soweto*.

4. Wits University Health Science Research Day, August 2008
   Ntyintyane, L., Panz, V.R., Raal, F.J. and Gill, G.V. *Postprandial hyperglycaemia in urbanized South African Blacks with and without coronary artery disease.*

LECTURES

1. Johannesburg Hospital, Endocrine Unit, CME Meeting, 2005
   Presenter: L. Ntyintyane
   Topic: Metabolic syndrome in black patients with documented coronary artery disease.

2. Wits University, School of Public Health, CME Meeting, 2007
   Presenter: L. Ntyintyane
   Topic: Metabolic syndrome: are we under-diagnosing it?

3. Wits University, Chemical Pathology Division, CME Meeting, 2008
   Presenter: L. Ntyintyane
   Topic: Cardiovascular risk factors in Soweto.

4. North West University, School of Nutrition, CME Meeting, 2008
   Presenter: L. Ntyintyane
   Topic: Metabolic syndrome

LIST OF CONTRIBUTORS

1. Professor Frederick Raal
2. Professor Karen Sliwa
3. Dr Vanessa Panz
4. Dr Geoff Gill (Liverpool School of Tropical Medicine, UK)
5. Zaiboonnisa Holland
6. Professor Jeff Wing
7. Robin Dolman

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LIST OF ABBEVIATIONS

AMI	acute myocardial infarction
ADA	American Diabetes Association
ATP III	Adult Treatment Panel III
AUC	area under the curve
CAD	coronary artery disease
CVD	cardiovascular disease
DM	diabetes mellitus
HDL	high density lipoprotein
HOS	Heart of Soweto Study
IGT	impaired glucose tolerance
TG	triglycerides
LDL	low density lipoprotein
OFTT	oral fat tolerance test
NGT	normal glucose tolerance
MS	metabolic syndrome
NCEP	National Cholesterol Education Program
WHO	World Health Organisation

KEY WORDS

postprandial glucose

epidemiological transition

urbanisation

coronary artery disease

metabolic syndrome

atherogenic dyslipidaemia

obesity

postprandial hyperglycaemia

inflammation

risk factors

diabetes mellitus

publications

ABSTRACT

In many developing countries with advanced stages of the nutrition transition, the burden of coronary artery disease (CAD) has shifted from the rich to the poor. Much of this transition is caused by changes in lifestyle, in particular: dietary changes, an increase in weight and obesity, a decrease in physical activity, high levels of stress, and increasing tobacco and alcohol consumption. However, we have come to appreciate a prominent role for inflammation in atherosclerosis and its complications.

Globalization, urbanization and Westernization of lifestyle will increase the socio-economic burden posed by non-communicable diseases in middle-to-low-income countries. In South Africa, it is mainly the African population that is experiencing rapid urbanization and the nutrition transition.

Reliable ischaemic heart disease (IHD) mortality data are not available for the black population of South Africa.

The purpose of this thesis was: to determine whether factors such as inflammation, postprandial lipaemia and hyperglycaemia are important determinants in black patients with documented CAD (with no previous known history of diabetes mellitus) and their age matched controls; to assess the prevalence of the metabolic syndrome (MS) in black patients and abnormal glucose regulation on black patients with CAD; and to compare the metabolic syndrome prevalence rates using the National Cholesterol Education Program Adult Treatment III (NCEP: ATP III) and International Diabetes Federation (IDF) definitions.

Socio-economic status, anthropometric data, glucometabolic variables, LDL particles and MS prevalence rates were measured using 40 patients and 20 controls. The patients were selected consecutively on the basis of a coronary angiogram performed during the preceding 24 months. All subjects had significant CAD, which was defined as more than 50% lesions in one or more major coronary arteries. Subjects with severe hypercholesterolaemia, defined as an untreated total cholesterol level over 7.5 mmol/l,

were excluded from the study. Those subjects with diabetes mellitus or HIV/AIDS were excluded from the study.

Paper 1, titled 'Metabolic syndrome, undiagnosed diabetes mellitus and insulin resistance are highly prevalent in urbanized South African blacks with coronary artery disease', demonstrated a high prevalence of MS in black patients with established CAD. To our knowledge, this is the first report from South Africa that documents the prevalence of the syndrome in black patients with CAD. Almost all of our patients had previously diagnosed hypertension (95%). The second most frequent risk factor was an elevated glucose concentration, which was seen in half the patient cohort. The importance of obesity, particularly abdominal obesity expressed as waist circumference (WC), is well documented as a risk factor for MS. An unexpected outcome of our study was that half of the patients had abnormal glucose regulation, despite the exclusion of previously diagnosed DM. This high prevalence was revealed by the oral glucose tolerance test (OGTT).

Paper 2 compares the MS prevalence estimates, as defined by NCEP: ATP III and IDF, amongst urbanized black South Africans with CAD. The IDF proposed a single unifying definition in 2005, as different definitions used different sets of criteria; this led to confusing and inconsistent estimations of MS prevalence. The new definition standardizes the criteria for the diagnosis of MS and offers a fresh assessment of the syndrome. The main findings that arose from the study were that both definitions generated similar prevalence estimates of MS and the two definitions similarly identified the presence or absence of MS in more than 80% of patients. This study demonstrated that postprandial lipaemia and hyperglycemia were common in black CAD patients. Small dense LDL particles were highly associated with CAD. Fasting triglyceride concentrations was the strongest determinant. Prolonged exposure of the endothelium to TG–rich atherogenic remnant particles might be the reason why postprandial increases in TG account for greater CAD risk.

Paper 3 assessed postprandial lipaemia in black CAD patients with and without metabolic syndrome. This study was the first to contribute information about postprandial lipaemia and hyperglycaemia in urbanized South African blacks with CAD. Fasting lipid profiles and postprandial responses to the oral fat load were similar

in patients with and without metabolic syndrome. A possible explanation might be that because patients in both groups had established CAD, they exhibited some of the underlying features of CAD, such as atherogenic dyslipidaemia. The main finding was that postprandial lipaemia was common in black CAD patients, including patients with metabolic syndrome. Fasting triglycerides concentration was the strongest determinant. Small, dense LDL particles were highly associated with CAD.

Paper 4 reports on the assessment of postprandial hyperglycaemia in urbanized blacks with and without CAD. Results showed that glucose AUC was significantly higher in the patients than in control subjects and 120 min. glucose, followed by 0 min. glucose concentration, were the strongest determinants of postprandial hyperglycaemia. Our study demonstrated that as glucose tolerance declined across the normal glucose tolerance, impaired glucose tolerance and diabetes mellitus categories, peak glucose concentrations occurred later in the oral glucose tolerance test; insulin and proinsulin responses were also delayed. A comparison between CAD patients and control subjects drawn from the same ethnic population verified that abnormal glucose tolerance and insulin resistance were more prevalent in the patients with CAD.

Paper 5 aimed at investigating whether carotid intima-media thickness (CIMT) is a predictor of CAD in South African black patients. The results showed that CIMT correlated with evidence of angiographically proven CAD. The findings of this study need to be considered within the context of its limitations, i.e. the low number of women and some bias towards only hospital referred CAD patients. It was not our intention to recruit more men than women, but because CAD is more prevalent in men, the majority of participants happened to be male. Performance of the OGTT and hyperinsulinaemic euglycaemic clamp technique is time consuming and requires considerable laboratory resources; therefore a relatively small number of patients and control subjects were studied. These limitations do not detract from the overall conclusions.

Paper 6 evaluated markers of inflammation in black CAD patients, some of whom had MS. Leptin was the only marker that increased with additional MS criteria. Elevated hs-CRP concentrations indicated an inflammatory state in CAD patients. Association of

leptin with BMI, waist circumference (WC) and hs-CRP revealed a close link with MS, obesity and inflammation in urban black South African CAD patients.

Paper 7 investigated the role of diet, socio-demographics and physical activity in a black South African population with CAD, compared to a healthy control group. While diet is known to be affected by urbanisation, differences in dietary intake were observed between the two urban groups, despite the similarity in their socio-demographic profile.

The study highlighted the clinical relevance of MS, its likely impact on morbidity and mortality, and that its identification is, therefore, important in risk assessment of patients with CAD. Increasing recognition of MS is, therefore, an initial step in addressing the metabolic problems associated with the syndrome. Furthermore, it was shown that a preponderance of small, dense LDL particles was highly associated with CAD in black patients. Although CAD prevalence is still low at this stage, it is likely to increase rapidly among urban dwellers as they adopt a Western lifestyle.

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CHAPTER 1: INTRODUCTION

1.1 HYPOTHESES / RESEARCH QUESTIONS

1. Is there a difference in the biochemical risk factor profile of black subjects with coronary heart disease (CAD), compared to age matched black controls without CAD?
2. What are the prevalence / incidence rates of diabetes, insulin resistance and the metabolic syndrome in SA presenting with CAD?
3. Are there post prandial glucose differences in the black CAD subjects compared to the age matched controls without CAD?
4. Are there post prandial lipid differences in the lipids and lipoproteins of black CAD subjects compared to the age matched controls without CAD?
5. Is Carotid Intima Media Thickness (CIMT) a predictor of CAD in South African black patients?
6. What is the influence of social factors and lifestyle on the onset of CAD?

1.2 RATIONALE OF THE STUDY

Rapid economic development and the consequent improvement in living conditions, nutrition, and health care have resulted in declines in infant mortality and deaths from infectious diseases and therefore in increases in life expectancy in many developing countries including China. (1) In 2001, cardiovascular disease (CVD) was already the No.1 cause of death worldwide, yet little global attention has been to the challenge of reducing this burden in developing countries, where it is on the rise. (2) In 2006-2007 the World Health Organization (WHO) allocated 87 % of its total budget to infectious diseases, 12 % to non-communicable diseases, and less than 1 % to injuries and violence. (3) CVD and other chronic diseases remain excluded from the United Nation's Millennium Development Goals (MDG) three infectious diseases – tuberculosis, human immunodeficiency virus (HIV) infection or the acquired immunodeficiency syndrome (AIDS), and malaria –have attracted the greatest attention from international donors, but together they are responsible for only 10 % of the deaths

in the world. (4) If nothing is done to reduce the risk of chronic diseases, an estimated US $ 84 billion of economic production will be lost from heart disease, stroke, and diabetes alone between 2006 and 2015. (5) This will be principally due to decline in deaths occurring in infancy, childhood, and adolescence and is related to more effective public health responses to perinatal, infectious and nutritional deficiency disorders, and to improved economic indicators such as per capita income. (6) Recent lifestyle changes in developing countries (7) and urbanization have resulted in the view that coronary heart disease will become the leading cause of death worldwide. (8) The epidemiological transition in turn, is predicted upon demographic and nutritional transitions occurring as a result of socio-economic development and increasing globalization that alter the determinants and risk factors for CVD and hence the patterns of CVD. (9)

There are very few studies at all that specifically report on the form, characteristics and extent of CAD among Black Africans . In black Africans, CAD has, until recently, been rather uncommon. (10) However, although still relatively uncommon, the incidence is thought to be increasing, due to changing socio-economic conditions. (11) In 1959 and 1960, an autopsy study at the Baragwanath Hospital (CHBH) in Soweto, South Africa, reported only one case of CAD; in 1976, fourteen cases were reported. (12) Historically, clinical coronary artery disease has also been remarkably rare in black South Africans. Records from South Africa's largest hospital, the CHBH showed that in the early 1990s, on average, approximately 70 black patients with CAD were admitted annually. (13)

A recent review of patients who underwent coronary angiography at CHBH revealed that in 2002 the number with significant CAD had increased somewhat, to 85 patients.

Presently approximately 150 patients are admitted annually to CHBH with acute coronary syndromes (Sliwa). However, there are few empirical studies on the changing profile of CVD in black Africans, especially those in urban settings. White South Africans have rates of CAD that are amongst the highest in the world and South Africans of Indian descent also have high CAD rates. The increase in the volume of cases has been far less than expected, however, and CAD remains an unusual cause of admission in black patients.

Table 1: Leading cause of death in 2001

	Low and middle income countries			High income countries		
	Cause	Deaths (millions)	% of total deaths	Cause	Deaths (millions)	% of total deaths
1	Ischaemic heart disease	5.70	11.8%	Ischaemic heart disease	1.36	17.13%
2	Cardiovascular disease	4.61	9.5%	Cardiovascular disease	0.78	9.9%
3	Lower respiratory infections	3.41	7.0%	Trachea, bronchus, lung cancers	0.46	5.8%
4	HIV/AIDS	2.55	5.3%	Lower respiratory infections	0.34	4.4%
5	Perinatal conditions	2.49	5.1%	Chronic obstructive pulmonary disease	0.30	3.8%
6	Chronic obstructive pulmonary disease	2.38	4.9%	Colon and rectum cancers	0.36	3.3%
7	Diarrhoeal diseases	1.78	3.7%	Alzheimer's disease and other dementias	0.21	2.6%
8	Tuberculosis	1.59	3.3%	Diabetes mellitus	0.20	2.6%
9	Malaria	1.21	2.5%	Breast cancer	0.16	2.0%
10	Road traffic accidents	1.07	2.2%	Stomach cancer	0.15	1.9%

Ten leading causes of death by income groups, 2001

Lancet 2006; 367: 1747-57

This low prevalence of CAD prevails in South Africa despite considerable Westernization of lifestyle and the accompanying rise in risk factors for CAD, such as obesity, atherogenic dyslipidaemia, hypertension and type 2 diabetes mellitus (DM). (14) However, there are few empirical studies on the changing profile of CVD in black Africans, especially those in urban settings. (15) Furthermore, there are very few studies at all that specifically report on the form, characteristics and extent of CAD among this group. (16) As noted above, historically, CAD amongst black Africans is indeed uncommon.

Increasing rates of CAD amongst this group have been observed and recently reported.

A large body of epidemiological studies, including the Interheart study, clearly demonstrated the emergence of cardiovascular disease epidemic in low-to-middle income countries. Ischaemic heart disease and cerebrovascular disease (stroke) were leading cause of death in high income and low-and-middle- income countries (see Table 1 above). (17)

Data presented in the report "Chronic Diseases of Lifestyle in South Africa 1995 – 2005", shows that most of the South African population is at risk of a chronic disease profile related to the Western lifestyle. (18) In the Interheart study, recently published in the Lancet, a standardized case control of similar cohorts in 52 countries examined the importance of risk factors for CAD on a worldwide basis. (19) On an adjusted basis, the following five modifiable risk factors were found to be most predictive of this event: smoking, raised apoB/apoA1 ratio, diabetes, hypertension and obesity. (20)

Global trade and marketing developments are driving the nutrition transition towards diets with a high proportion of saturated fat and sugars. This diet, in combination with tobacco use and little physical activity, leads to population-wide atherosclerosis. The epidemiological transition, change in the metabolic cardiovascular risk factors and the emerging coronary artery disease within the black population have been not receive the necessary attention, until thus far. South Africa cannot escape the public health impact of globalization and urbanization.

1.2.1 Metabolic risk factors

The constellation of the metabolic abnormalities comprising central obesity, raised triglycerides (TG), reduced high-density lipoprotein (HDL) cholesterol, raised blood pressure (BP) and raised fasting glucose is referred to as metabolic syndrome (MS). (21) In the past decade, several panels of experts have attempted to provide a definition of the diagnostic criteria for MS. The WHO published the first definition in 1998; this was soon modified by the European Group for the Study of Insulin Resistance. In 2001, the NECP: ATPIII issued its definition. (22) Subsequently, the American College of Endocrinology released a position statement on MS. Each of these definitions used differing sets of criteria, which led to confusing and inconsistent estimations of the prevalence of MS.

Table 2: Current definitions of Metabolic Syndrome

WHO 1999	EGIR 1999	ATPIII 2001	IDF 2005
Diabetes or impaired glucose tolerance or insulin resistance*	Insulin resistance* or hyperinsulinaemia (only non-diabetic subjects)	3 or more risk factors	Central obesity Waist circumference[$†] - ethnicity specific
Plus 2 or more of the following:	Plus 2 or more of the following:	Plus 3 or more of the following:	Plus any two of the following:
1. Obesity: BMI>30 kg/m^2 or Waist Hip ratio >0.9 (M) >0.85 (F) 2. Dyslipidaemia: Triglycerides ≥1.7 mmol/l or HDL-C <0.9 mmol/l (M) <1.0 (F) mmol/l 3. Hypertension: Blood pressure ≥140/90mmHg or medication 4. Microalbuminuria: Albumin excretion ≥20 µg/min	1. Central obesity: Waist circumference ≥94cm (M), ≥80cm (F) 2. Dyslipidaemia: Triglycerides >2.0 mmol/l or HDL-C <1.0 mmol/l 3. Hypertension: Blood pressure ≥140/90 mmHg and/or medication 4. Fasting plasma glucose ≥6.1mmol/l	1. Central obesity: Waist circumference >102cm (M), >88cm (F) 2. Hypertri-glyceridaemia: Triglycerides ≥1.7 mmol/l 3. Low HDL-C: <1.0 mmol/l (M), <1.3 mmol/l (F) 4. Hypertension: Blood pressure ≥130/85mmHg or medication 5. Fasting plasma glucose ≥6.1 mmol/l	1. Raised triglycerides≥ (1.7 mmol/l) or specific treatment for this lipid abnormality 2. Reduced HDL-C < 1.03 mmol/l (M) <1.29 mmol/l (F) or specific treatment for this abnormality 3. Hypertension: 130/85mmHg or medication 4. Fasting plasma glucose ≥5.6 mmol/l or previously diagnosed type 2 diabetes[^]

In response to this controversy, the International Diabetes Federation (IDF) proposed a single unifying definition in 2005. (23) The focus of the new definition is central obesity, (24) as assessed by waist circumference (see Table 2 above). It varies from the earlier NECP: ATPIII definition in that it incorporates specific waist circumference (WC) cut-offs for men and women from different ethnic groups.

While the pathophysiology of MS and each component is not fully understood, obesity and insulin resistance are thought to be important causative factors. (25)

Abdominal obesity is the form of obesity most strongly associated with MS; it presents clinically as increased WC. Atherogenic dyslipidaemia describes the combination of raised TG and reduced concentrations of HDL cholesterol. (26) Hypertension, often regarded as being less 'metabolic' than other components, usually coincides with obesity and commonly occurs in insulin–resistant individuals. Insulin resistance is present in the majority of patients with MS, many of whom develop impaired glucose tolerance (IGT). (27) When IGT progresses to DM, an elevated glucose concentration constitutes a major independent risk factor. The increased risk for CAD, however, is already present at modestly elevated levels of blood glucose that are well below the threshold for the diagnosis of DM. (28) Indeed, a recent survey done across Europe on CAD patients without previously diagnosed DM reported a high prevalence of both IGT (36%) and newly detected DM (18%). (29) The conceptual importance of the MS is, therefore, to indicate that these various factors are more likely to occur together rather than separately; when they cluster, they are related to increased risk of CVD morbidity and mortality.

Much interest is currently being focused on the striking global increase in the prevalence of MS. However, although it is acknowledged that the overall prevalence of MS is increasing globally, the rates vary widely among different populations across the world. Japan has one of the lowest prevalence rates (only 6%), followed by 11% in Finland, 27% in Mexico and 32% in India. (30) Prevalence is higher in the USA, where rates of 34% and 39% were reported for black and white Americans respectively. In Africa, an even higher prevalence rate of 43% was found in black Zimbabweans. (31) However, there remains a paucity of information about the prevalence of MS and abnormal glucose regulation in the inhabitants of sub-Saharan Africa.

One of the main aims of this thesis was to assess the prevalence of MS in the South African emerging black population with established CAD. An additional aim was to assess the prevalence of abnormal glucose regulation in these patients, who had no previously known DM.

1.2.2 Lipids

The role of lipids and lipoproteins in the pathogenesis of atherosclerosis was first described more than 50 years ago. Dyslipidaemia is a major, if not the major, risk factor for CAD. An elevated LDL-cholesterol level appears to be the primary CAD risk factor, as some elevation of LDL-cholesterol seems to be necessary for the development of CAD. Lowering LDL cholesterol has now been conclusively shown to decrease CAD events as well as total mortality. (32)

Although increased LDL cholesterol appears to be the primary CAD risk factor, small dense particles in the LDL sub-fraction are particularly atherogenic and have also been implicated in atherogenesis. (33) Small, dense LDL particles are more susceptible to oxidation than large, buoyant LDL particles and they are retained to a higher degree in the arterial wall. Furthermore, small, dense LDL particles display reduced binding to LDL receptors and remain in the circulation system for longer periods of time. These LDL particles are, therefore, subject to a greater degree of structural modification, which, in turn, may increase their atherogenic potential. (34)

Elevated fasting triglyceride levels also appear to be an independent risk factor for CAD in epidemiological studies. (35) HDL-cholesterol, on the other hand, mediates reverse cholesterol transport and is a negative risk factor for CAD. (36) However, the measurement of serum lipids and their carrier lipoproteins, such as apolipoprotein B and A1, cannot identify all patients with CAD. (37)

In addition, studies by Fraser and Zilversmit in the 1970s led to the postprandial theory of atherosclerosis. (38) This hypothesis proposed that atherosclerosis is a postprandial phenomenon that largely depends on the metabolic response to the ingestion of food. The number of meals consumed and the long duration of postprandial lipaemia lead to a continual fluctuation in the degree of lipaemia throughout the day. Evidence is accumulating that many patients with established CAD also have postprandial abnormalities in lipid metabolism. This relationship has been reported by several researchers.

All of these studies showed that CAD patients had elevated TG concentrations as well as a prolonged postprandial response after a fatty meal. Postprandial lipaemia is characterized by a marked increase in plasma levels of TG and TG–rich lipoproteins. This is accompanied by a reduced concentration of high density lipoprotein (HDL) cholesterol and a modestly raised level of low density lipoproteins (LDL) cholesterol. (39)

An enhanced TG rise postprandially also occurs in other conditions that are associated with an increased risk of vascular disease, such as obesity, hypertension, DM and the metabolic syndrome. In fact, postprandial lipaemia is now considered to be another characteristic feature of MS. A further link between postprandial lipaemia and MS is that an elevated fasting TG concentration is accompanied by abdominal obesity. These two risk factors, together with hypertension, low HDL cholesterol and raised plasma glucose concentrations, are diagnostic criteria for this syndrome.

Evidence is accumulating that many patients with established CAD also have postprandial abnormalities in lipid metabolism (40); this relationship has been reported by several researchers. All of these studies showed that CAD patients had elevated TG concentrations as well as a prolonged postprandial response after a fatty meal.

The relationship between insulin resistance and CAD and its risk factors has only been investigated occasionally in a black African setting. (41) Patients with CAD tend to be insulin resistant and display delayed and higher peak plasma triglyceride levels after a fat load. (42) Insulin resistance and postprandial lipaemia may, therefore, be important risk factors for CAD. Relatively little is known, however, about the occurrence of postprandial lipaemia in urbanized black South Africans.

1.2.3 Inflammation, atherosclerosis and CAD

Atherosclerosis is a multifactorial disease for which numerous pathogenetic concepts have been proposed. (43) Many patients with coronary atherosclerosis lack conventional risk factors, suggesting that there are additional, unidentified factors that contribute to vascular injury. (44) For example: evidence indicates that microorganisms may play a role in the pathogenesis of the disease. Epidemiological studies indicate that

infectious agents may predispose patients to atherosclerosis and its adverse clinical events. (45)

Inflammation also appears to play an important role in the onset development and evolution of atherosclerotic lesions. Laboratory and pathological data support the idea that inflammation has a role in both the initiation and progression of atherosclerosis, whilst chronic inflammation of the artery wall is a principal driving force behind the development of atherosclerosis. (46)

Infections may predispose to atherosclerosis by inflicting endothelial injury. Organisms implicated are intracellular pathogens, including viruses such as: cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1) and hepatitis A virus.

Virtually all regional acute myocardial infarcts are caused by thrombosis developing on a culprit coronary atherosclerosis. Vascular cell adhesion molecule-1 (VCAM-1) binds precisely the types of leukocytes found in early human and experimental atheroma, the monocyte and T lymphocyte. (47)

Thrombus formation is the proximate cause of myocardial infarction, but atherosclerosis, the chief underlying cause, is a chronic disease that progresses over decades. Endothelial dysfunction is one of the earliest manifestations of atherosclerosis. (48)

A number of epidemiological studies have shown that the acute phase reactant, CRP, is an important risk marker for atherosclerosis and coronary heart disease. Elevated serum levels of CRP have been widely considered to be non-specific but sensitive markers of acute inflammatory response. (49) However, there are few data to indicate whether inflammation increases the risk of myocardial infarction, stroke and venous thrombosis.

The mechanism that relates the level of CRP to atherothrombosis remains unclear.

1.2.4 Nutrition and CAD

The major cardiovascular diseases have at their core atherosclerosis and hypertension, both of which are profoundly affected by diet. (50) Prospective studies suggest that a high trans fat intake is associated with an increase in heart disease rates. (51) Increased consumption of fruit and vegetables has been shown to be associated with a reduced risk of stroke in most epidemiological studies. (52)

Schutte et al, (53) have shown that the underlying cause of the decreased health status of black people is due to the Western lifestyle adopted in an urban environment. Urbanization and the nutrition transition in South Africa are accompanied by an increase in the CAD risk factors in Africans. (54) During the period 1993 - 1996, the percentage of urbanized black South Africans increased from 35.8 to 43.3%. (55)

CAD is a major health problem in all population groups of South Africa. Apart from the black South Africa population, epidemiological transition has been found in the Chinese. (56) Vorster et al, (57) demonstrated the nutritional transition in black South African population groups is due to: a decreased intake of the staple food, maize porridge; and an increased intake of animal derived foods. This has resulted in increases in serum total cholesterol, LDL cholesterol and plasma fibrinogen. All are accepted risk factors of cardiovascular diseases that can be influenced by diet.

It is commonly believed that an increased intake of total dietary fiber reduces postprandial glucose responses and lowers total and LDL cholesterol levels. (58) A diet high in total dietary fiber and the consumption of a soluble dietary fiber significantly decreased levels of the inflammatory marker C- reactive protein.

Foods of high density are often selected by the poor because of cost and availability, whereas fruit and vegetables are not readily available to the poor.

1.2.5 Intima-media thickness, atherosclerosis and CAD

Atherosclerosis is a generalized disease of the arterial wall, which may progress or regress depending on a plethora of factors. (59) Atherosclerosis is a protracted and lifelong progressive disease of the arterial wall that leads to cardiovascular disease.

Thickening of the intima-media is commonly recognized as the initial stage in the development of atherosclerosis. (60) CIMT is an intermediate phenotype for early atherosclerosis. (61) Traditional risk factors, such as male gender, ageing, high blood cholesterol, being overweight, elevated blood pressure, diabetes and insulin resistance and cigarette smoking are positively associated with carotid IMT in observational and epidemiological studies. (62)

Imaging progression and regression of atherosclerosis might be used as a surrogate marker for cardiovascular disease occurrence. (63) Common and internal carotid IMT can be viewed as an estimate of atherosclerosis quantity. Intima-media thickness of large artery walls, especially carotid, can be assessed by B-mode ultrasound in a relatively simple way and represents a safe, inexpensive, precise and reproducible measure. Vascular markers of atherosclerotic burden allow atherosclerosis to be studied in the sub-clinical phase of the disease and facilitate the assessment of new therapies for modifying coronary heart disease risk factors. (64) A number of longitudinal studies have examined the relationship between IMT and future events, mostly frequently the incidence of cardiac events and cerebrovascular events. No studies have been done on the South African black population with coronary artery diseases.

1.3 LITERATURE REVIEW OF CORONARY ARTERY DISEASE IN AFRICA

Opinions and reports suggest that as long as Africa remains impoverished it is unlikely that CAD will emerge as a significant health problem. The Interheart Africa study, which examined the impact of modifiable CAD risk factors on myocardial concluded that there were low numbers of myocardial infarction cases. In Kenya there was one case of autopsy confirmed myocardial infarction among 2000 adult admissions at a large regional hospital in 1960. (65) Analysis of the records of 3645 autopsies performed in Accra in Ghana over a 33 year period (1921 - 1953) revealed only thirty cases of coronary heart diseases.

However, with growing urbanization and economic development, the rates may increase and resemble those seen in South Africa. (66) Several studies have shown that urbanization and nutrition in South Africa is accompanied by an increase in the CAD

risk factors in Africans. Urbanization is, of course, linked with economic development and education level, being the main reasons why people move from under-developed, rural areas with limited job and education opportunities to more developed urban areas.

In South Africa, recent surveys have confirmed both the high prevalence of hypertension among Zulu and Xhosa subjects and an urban rural gradient. In a study, done at Johannesburg Hospital, Seftel and Kew found that a total of 24 black patients with myocardial infarction were admitted during the 4 year period 1965 - 1968. These authors concluded that the prevalence had increased as a result of a Western life style in an urban environment. (67)

The THUSA (Transition and Health during Urbanization of South Africans) study was a cross sectional epidemiological study that looked at how the factors associated with urbanization influences the cardiovascular system as a whole. One of its conclusions was that factors associated with urbanization include elevated blood pressure. A classic rise in blood pressure was observed in the African male who had transversed from a monoculture, rural environment to a multi-cultural urban environment. The urban group also had a higher abdominal fat content.

Lipid levels increase with urbanization in both men and women. The BRISK study (68) conducted by Krisela Steyn (Medical Research Council (MRC) in the Cape Peninsula) also examined the risk factors for coronary heart disease in black populations. This was a cross sectional study of 986 black people aged 15 - 64 years, living in the Cape Peninsula. Findings included: with rapid urbanization of the black population there is a concern over a concomitant adoption of the lifestyles of typical, industrialized populations; this in turn raises the possibility of increased mortality from IHD and CVD in the future.

The overall risk profile identified in the BRISK study shows that the black, male, urban population of the Cape Peninsula already has considerable IHD and about 30.8% of males aged 25 years and above have at least one risk factor. (69) The high prevalence of obesity among older black women, (70) has partially contributed to the development of hypertension in this group. (71)

The Interheart Africa study remains the only major epidemiological study of note done on coronary artery disease in Africa: 578 acute MI cases from nine sub-Saharan African countries were recruited in a period of 4 years (1999 - 2003). The Interheart Africa data showed for the first time that only five risk factors account for 89.2% of the risk for MI, i.e.: tobacco smoking, hypertension, diabetes, abdominal obesity and lipoprotein ApoB/ApoA-1.

Consequently, the data from this report contradicts the theory that black African people with exposure to the known cardiovascular risk factors are immune to developing AMI. (72) Unhealthy lifestyles (73) and the resultant emerging CVD risk factors impart at least the same level of risk for AMI (74) as that found in the overall INTERHEART study. The major limitation of the INTERHEART study was the small sample of black African myocardial infarction cases.

I deliberately chose to exclude the PURE and HEART Of Soweto Studies. It would be too premature to comment on them as they are still on the infancy stages. Another study worth mentioning is the Agincourt surveillance by the MRC/WITS Rural Public Health and Health Transitions Research Unit since 1992. (75) The research findings on stroke and cardiovascular disease indicate a prevalence of hypertension in men and women; pronounced obesity in women; more frequent smoking in men than women and modest cholesterol and high HDL-cholestrol concentrations. The studies are still on going. However the above studies, do not fully address the question, what do we know about the burden of cardiovascular disease in South Africa?

CHAPTER 2: RESEARCH DESIGN

This was a retrospective study. Forty black patients (33 males and 7 females) with angiographically confirmed CAD were recruited over 24 months. All the patients had significant CAD, which was defined as more than 50% lesions in one or more major coronary arteries demonstrated on coronary angiography. Twenty subjects (13 men and 7 women), who were matched with the patients for age, body mass index (BMI), waist circumference and waist to hip ratio were used as controls. Patients and control subjects were classified according to the number and extent of lesions in coronary arteries, which were verified by a coronary angiogram performed in the preceding 24 months. None of the control subjects had evidence of coronary atherosclerosis on coronary angiography. Patients who had a previous myocardial infarction (MI) were at least three months post-MI before the study started. The majority of patients and control subjects had moderate hypertension and they were taking anti-hypertensive medication at the time of the study.

Patients who had a dominant risk factor, such as severe hypercholesterolaemia or previously diagnosed DM, were excluded from the study. Other exclusion criteria were: HIV-positive status, overt liver, renal or thyroid disease and smoking more than 20 cigarettes per day.

On the first day of the study, each subject underwent a structured examination, which included an interview, height, weight and waist measurements, a fasting venipuncture and an oral glucose tolerance test. Height and weight were measured to the nearest 0.5 cm and 0.1 kg respectively. Body mass index was calculated as weight divided by height squared. Waist circumference was determined to the nearest 0.1 cm using a measuring tape positioned at the midpoint between the lowest rib and the iliac crest.

Questionnaires were used to obtain information about demographic variables, medical history, medication use, dietary habits, physical activity and smoking status (see Appendix A). On the second occasion, after an overnight fast, the hyperinsulinaemic euglycaemia clamp technique was performed. An eight-hour oral fat tolerance test was

done on the third visit, after the subject had undergone a 12 hour overnight fast. All participants provided written, informed consent to take part in the study, which was approved by the Human Research Ethics Committee of the University of the Witwatersrand (see Appendix B).

2.1 SUBJECTS

Black patients attending the Chris Hani Baragwanath Hospital in Soweto, Johannesburg Hospital and Helen Joseph Hospital, who had undergone diagnostic coronary angiography to confirm or exclude CAD, were approached to participate in the study. Forty patients (33 men and 7 women) with documented CAD, agreed to take part. The CAD patients were all attending cardiac clinics in the above-mentioned hospitals. The fact that there were more men than women was pure coincidence and not studies bias. Patients were chosen on first come, first served basis. Newly diagnosed CAD patients were identified and recruited into the study. It just happened that there were more men than women admitted for CAD during the study period. The majority of the study patients were recruited from Baragwanath hospital cardiac High Care ward. Twenty control subjects (13 men and 7 women), who were matched with the patients for age, Body Mass Index (BMI), Waist Circumference (WC) and waist-to-hip-ratio (WHR) were also studied. The control subjects were also cardiac patients with no documented history of CAD. Some of them were admitted for valvular or dilated cardiomyopathy related pathologies.

Patients and control subjects were classified according to the number and extent of lesions in coronary arteries, which were verified by a coronary angiogram performed in the preceding 24 months. All of the patients had significant CAD, which was defined as more than 50% lesions in one or more of the major coronary arteries. None of the control subjects had evidence of coronary atherosclerosis on coronary angiography. Patients who had a previous MI were also at least three months post-MI before the study started. The majority of patients (95%) and control subjects (75%) had moderate hypertension and they were taking anti-hypertensive medication at the time of the study.

2.2 EXCLUSION AND INCLUSION CRITERIA

Subjects must have a blood pressure of < 160/100 mm/Hg for inclusion into the study. Patients who had a dominant risk factor, such as severe hypercholesterolaemia, defined as an untreated total cholesterol of over 7.5 mmol/l, or subjects with clinical features of familial hypercholesterolaemia, were excluded from the study. Subjects who had been diagnosed previously with diabetes mellitus were excluded from the study. The potential recruits were asked about their past medical history and medications to determine if they were diabetic or not. HIV positive subjects were excluded. Most of the subjects were not keen to reveal their HIV status. Hospital records proved useful in this regard. Other exclusion criteria were smoking more than 20 cigarettes per day, overt liver, renal or thyroid disease.

All subjects gave written, informed consent to participate in the study, which was approved by the Human Research Ethics Committee of the University of the Witwatersrand (see Appendix A).

Procedure

Full explanation was given to the study subjects during the recruitment phase. This was done in a language preferred by the study subjects. Those subjects who agreed to partake in the study will give a verbal and written consent. Lipid lowering drug therapy (statins and fibrates) were stopped 4 weeks prior to testing. Other drugs which may alter lipid levels and insulin resistance (e.g. thiazide diuretics, beta-blockers, steroids etc) as well as aspirin were stopped 3 days prior to testing. Following a 12-hour overnight fast, subjects had baseline assessments performed followed by an oral glucose tolerance test. On the day of the study, each participant underwent a structured exam, which included an interview. Height, weight, WC, WHR measurements were done. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. BMI was calculated as weight (kg) divided by height (m) squared. WC was determined to the nearest 0.1 cm using a measuring tape positioned at the midpoint between the lowest rib and the iliac crest and hips were measured at the largest gluteal circumference. These measurements were used to calculate WHR. Questionnaires were used to obtain information on

demographic variables, medical history, medication use, dietary habits physical activity and smoking status. The whole tests will take three weeks to complete. The first visit on week 1 was for the OGTT. A week later, the subject will return for the eight hourly Oral Fat Tolerance Test. the last week was for the insulin clamp.

2.3 METABOLIC STUDIES

2.3.1 Oral glucose tolerance test (OGTT)

A standard OGTT (75g glucose), as described by the WHO, was performed and glucose concentrations were measured at baseline and at two hours post-load. IGT and diabetes mellitus (DM) were defined according to the criteria adopted by the American Diabetes Association (ADA).

Normal glucose tolerance (NGT) was defined as a fasting glucose level of < 5.6 mmol/l and a two hour post load glucose level of < 7.8 mmol/l; alternatively a two-hour post-load glucose concentration of > 7.8mmol/l but < 11.1 mmol/l denotes an Impaired Glucose Tolerance (IGT). DM was diagnosed if the fasting plasma glucose value was >7.0 mmol/l or the two hour post load glucose value was >11.1 mmol/l.

2.3.2 Oral fat tolerance test (OFTT)

After a 12 hour overnight fast, an intravenous catheter was inserted into a forearm vein for blood sampling. The OFTT was performed as described by Patsch et al (76), with a slight modification of the fatty meal. Briefly it consisted of cream, chocolate-flavoured syrup, sugar and powdered milk, which provided energy from fat (83.5%), carbohydrates (14%) and protein (2.5%). The meal was consumed within 20 minutes, after which the participants were instructed not to take anything orally for the next eight hours, except for water. Lipid profiles compromising TC, HDL cholesterol, LDL cholesterol and TG concentrations were measured at baseline (defined as the value at 0h) and at 2 h, 4 h, 6 h and 8 h post-load. In addition, fasting levels of Apo A-1, Apo B, LP (a), free fatty acids (FFA), glucose and LDL particle sizes were measured.

2.3.4 Hyperinsulinaemic euglcaemic clamp technique

There is general agreement that the glucose clamp technique, particularly in its euglycaemic version, is the best available standard for the measurement of insulin action (how to measure insulin sensitivity). The clamp technique allows for the assessment of three important physiological variables. It provides a measure of the total amount of glucose metabolized, the response of the beat-cell can be quantified and the early and late phases of insulin secretion examined. (77) Briefly, an intravenous catheter was inserted by venipuncture for purposes of the infusion of glucose and insulin. A second catheter was inserted into a dorsal hand or wrist vein and the hand was enclosed in an electric heat pad, which was warmed to approximately 70 C to arterialize the blood obtained for all the samples. Three blood samples were drawn at 10-minute intervals to assess the basal glucose concentration. Thereafter, the glucose concentration was measured and the exogenous 10% dextrose infusion rate was adjusted at 10-minute intervals, as calculated by a computerized algorithm based on the negative feedback principle. Insulin-mediated glucose disposal was measured at a single infusion rate of 40 mU/m2/minutes (Actrapid, Novo Nordisk, Denmark). Blood glucose was clamped at 5.1 mmol/l for the next 120 minutes by infusion of 10% glucose at various rates, according to blood glucose measurements performed at 10 minute intervals.

The mean amount of glucose infused during the last 30 minutes was used to determine the steady-state glucose requirements. Insulin-mediated glucose disposal (M-value) was expressed as mg/kg/min, with a normal value being above 5.0 mg/kg/min and a value below this level indicating insulin resistance. A minimal mathematical model of glucose kinetics is implemented on a laboratory minicomputer during clamp experiments. (78) The computer estimates the fractional disappearance rate of glucose and calculates the rate of exogenous infusion required to match the desired concentration (M). The rate of glucose infusion necessary to support the glucose level is an index of the sensitivity of overall glucose utilization to insulin. PACBERG, a new computer program estimates the insulin dependent increase in fractional disappearance rate of glucose. (79) In addition the program calculates the rate of exogenous glucose infusion, which must be infused to maintain the desired glucose concentration. The clamp technique affords a highly reproducible method of assessing beat-cell sensitivity to glucose as well as of

quantifying the amounts of glucose metabolized by the body in vivo. hypoglycemic reactions are prevented .

2.4 INTIMA-MEDIA THICKNESS

2.4.1 Ultrasound Technique

B-mode ultrasound measurement of the carotid intima-media thickness was carried out on all patients as a blind study: the operator was unaware of the outcome of the coronary angiography. A standardized ultrasound technique was used, using a Toshiba System: Nemio Model SSA-550A. The transducer frequency was set at 11MHz for all patients.

Measurements of the intima-media thickness were carried out at the optimum angle of interrogation (OAI), which allows visualization of the flow tip divider, the common carotid artery (CCA), external carotid artery (ECA) and the internal carotid artery (ICA) from a single selected angle of the carotid arteries at the bifurcation. Doppler was used to verify the identification of the ECA and ICA.

The IMT was measured when the two echogenic lines, representing the lumen-intima interface and the media-adventitia interface, were visualized over a length of ≥1cm. Measurements of the IMT were done manually, using the calliper markers of the ultrasound unit.

The IMT at the optimal angle of interrogation was measured as the area of maximum thickness at the near and far walls of the CCA, BIF and ICA bilaterally (a total of 12 sites). In cases where calcified plaque obscured the IMT in the bulb, one wall was measured; the thickest measurement in each segment was imaged and recorded as the final measurement. The mean maximum IMT was recorded as the CIMT. Images were stored on a magnetic optical disc as well as on thermal paper.

The CIMT was calculated as the average for 12 sites for all subjects; this was done using the Excel programme for Windows XP. Mean values for the CIMT > 0.8mm were classified as increased thickness.

2.5 STATISTICAL ANALYSIS

Data analysis was performed using the GB-STAT program (Dynamic Microsystems, Inc, Silver Spring, MD, USA), with a value of $P< 0.05$ considered significant. One way analysis of variance (ANOVA) for repeated measures within a group or ANOVA for completely randomized measures between groups was used to assess any differences between the means of the variables, as appropriate. Comparison of two groups was done by the Student's paired or unpaired t-test, and the Mann-Whitney U-test or the Wilcoxon Signed-Rank test for parametric or non-parametric data, respectively. Results are expressed as mean +/- SD or as proportion (%). The frequency of M-values above and below the normal level was compared using Fishers Exact test. Area under the curve (AUC) for serial measurements of glucose, insulin and proinsulin concentrations at 0 minutes and after the glucose load was calculated using the trapezoid rule. Linear regression analysis and correlation coefficient calculations were performed to reveal any significant determinants of postprandial hyperglycaemia.

CHAPTER 3: MANUSCRIPTS

3.1 MANUSCRIPT 1

(In print – Ntyintyane, L.M., Panz, V.R., Raal, F.J. and Gill, G.V. The metabolic syndrome using the National Cholesterol Education Program and International Diabetes Federation definitions among urbanized Black coronary artery disease. Journal of Endocrinology, Metabolism and Diabetes of South Africa, Vol 12(1) 2007:6-12.

The manuscript is attached as Annexure C.

3.2 MANUSCRIPT 2

(In print – Ntyintyane, L.M., Panz, V.R., Raal, F.J and Gill, G.V. Metabolic syndrome, undiagnosed diabetes mellitus and insulin resistance are highly prevalent in urbanized South African Blacks with coronary artery disease. Cardiovascular Journal of South Africa 2006; 17:7-12.

The manuscript is attached as Annexure D.

3.3 MANUSCRIPT 3

(In print – Ntyintyane, L.M., Panz, V.R., Raal, F.J. and Gill, G.V. Postprandial lipaemia, metabolic syndrome and LDL particle size in urbanised South African Blacks with and without coronary artery disease. Q J Med 2008:101; 111-119.

The manuscript is attached as Annexure E.

3.4 MANUSCRIPT 4

(In print – Ntyintyane L, Panz V, Raal F, Gill G. Postprandial hyperglycemia in urban South African Blacks with and without coronary artery disease. Metabolic Syndrome and Related Disorders 2008)

Title: POSTPRANDIAL HYPERGLYCAEMIA IN URBANISED SOUTH AFRICANS WITH AND WITHOUT CORONARY ARTERY DISEASE

The manuscript is attached as Annexure F.

3.5 MANUSCRIPT 5

(Accepted for publication - Cardiovascular Journal of Africa)

Title: CAROTID INTIMA-MEDIA THICKNESS IS A PREDICTOR OF CORONARY ARTERY DISEASE IN SOUTH AFRICAN BLACK PATIENTS.

A copy of the full manuscript is attached as Annexure G.

3.6 MANUSCRIPT 6

(Accepted for publication – Metabolic Syndrome and Related Disorders)

Title: LEPTIN, ADIPONECTIN AND HS-CRP IN RELATION TO THE METABOLIC SYNDROME IN URBAN SOUTH AFRICANS WITH AND WITHOUT CORONARY ARTERY DISEASE (CAD).

A copy of the full manuscript is attached as Annexure H.

3.7 MANUSCRIPT 7

MANUSCRIPT IN PREPARATION

(Preparation for submission to The South African Journal of Clinical Nutrition)

Title: DIETARY INTAKE, SOCIO-DEMOGRAPHICS AND PHYSICAL ACTIVITY OF A BLACK URBANISED SOUTH AFRICAN POPULATION WITH CORONARY ARTERY DISEASE COMPARED TO HEALTHY VOLUNTEERS.

The manuscript is attached as Annexure I.

PUBLICATIONS PUBLISHED (BUT NOT PART OF THE THESIS)

1. Sliwa K, Wilkinson D, Hansen C, Ntyintyane L, Tibazarwa K, Becker A, Stewart S. Spectrum of heart disease and risk factors in a black urban population in South Africa (the Heart of Soweto Study): a cohort study. *Lancet* 2008; **371**:915-922.

2. Ntyintyane, L.M. Metabolic Syndrome: Fact or Fiction. *SA Cardiology and Stroke Journal*, Summer 2008; 2:37-44.

CHAPTER 4: DISCUSSION OF MAJOR FINDINGS

4.1 METABOLIC SYNDROME

The series of papers on MS show that black South African patients with CAD have a high risk of MS. Abdominal obesity measured as waist circumference was the most important risk factor for the syndrome and, together with hypertension and elevated glucose concentrations, formed the most frequent risk factor combination.

Obesity and insulin resistance are significant, interrelated causes of MS. Previously undiagnosed IGT and DM were common abnormalities. The study highlights the clinical relevance of MS, its likely impact on the morbidity and mortality and that its identification is, therefore, important in risk assessment of patients with CAD. The investigations showed, in addition, that the two definitions of metabolic syndrome i.e. new IDF criteria and the older NECP: ATPIII, similarly identified the presence or absence of the MS in 83% of the patients. That both definitions generated similar prevalence estimates of MS implies that the predicted risk of developing CAD, DM and other adverse events, based on the new IDF definitions, is likely to be similar to that observed with the NECP: ATPIII definition. Regardless of which definition is used, the high prevalence rate indicates that large numbers of black South Africans with CAD are likely to have MS.

Can we then extrapolate from these findings, that metabolic syndrome; is a better diagnostic screening tool for cardiovascular disease and diabetes? Secondly, do we need another syndrome? These two central issues underline the current debate on the clinical relevance of metabolic syndrome.

A school of thought believes metabolic syndrome is an invention of the pharmaceutical industry that is being rushed through. There is much fundamental, clinically important and critically missing information that warrant a more serious examination of whether medical science is doing any good by drawing attention to and presumed disease that does not stand on firm ground. (80) Such issues as follows: are all risk factors equally

important? Is the syndrome better than the Framingham risk score in cardiovascular disease screening? What is the underlying pathology?

P Zimmet and George Alberti the leading proponents in this regard, hold the opposing view that: "recognizing the syndrome provides a simple public health strategy to define those at high risk". (81) Lack of clear pathophysiology, is not an excuse to disregard its clinical importance. Although the aetiology of the syndrome remains uncertain, strong hypotheses implicate central adiposity, insulin resistance and low-grade inflammation. The syndrome is not intended to give an absolute risk of cardiovascular disease or diabetes but to highlight people at increased relative risk on whom doctors can then focus. In the Prospective Study of Pravastatin in the elderly at Risk (PROSPER), metabolic syndrome was not associated with increased risk of cardiovascular disease in those without baseline disease, but was associated with increased of diabetes. (82) In the 10-year Hoorn study, the metabolic syndrome, however defined, is associated with an approximate 2-fold increased risk of incident cardiovascular morbidity and mortality in a European population. (83) In conclusion, metabolic syndrome is not a substitute for the Framingham, but to complement it. Nonetheless, more research needs to be done on this area. Because prevalence of obesity and the metabolic syndrome is rapidly increasing in developing countries, leading to increased morbidity and mortality due to type two diabetes mellitus and cardiovascular disease. (84)

4.2 POSTPRANDIAL GLUCOSE

Worldwide, the number of people with prediabetes estimated at 314 million and projected at 418 million by 2015. (85) As the prevalence of and progression to diabetes continue to increase, diabetes related morbidity and mortality have emerged as major public health care issues. Abnormal glucose tolerance is almost twice as common amongst patients with a myocardial infarction. Little has been done on postprandial state in urbanized black patients with CAD, i.e. the period that compromises and follows a meal. It was hypothesized that postprandial hyperglycaemia is a CAD risk factor.

The study showed that postprandial hyperglycaemia is common in black South African CAD patients. Postprandial hyperglycaemia is determined by many factors, including

the timing, quantity and composition of a meal. High-calorie foods that are rich in refined carbohydrates can cause an exaggerated increase in blood glucose,
Which is repeated many times each day?

Recognition is growing that hypergylcaemia, and specifically postprandial hyperglycaemia, is a clinically significant risk factor for the development of coronary artery disease. The research results showed that glucose AUC was significantly higher in the patients than in control subjects at both 0 minutes and 120 minutes. The precise mechanisms through which postprandial hyperglycaemia exerts its adverse effects have not yet been fully elucidated, but some putative cause-effect interactions have.

Importantly, the time courses of postprandial hypergylcaemia and postprandial lipaemia are different: postprandial hypergylcaemia is typically an early phenomenon, followed by a prolonged period of postprandial lipaemia. Both processes, however, induce oxidative stress, which may cause endothelial dysfunction and which promotes the development of CAD.

4.3 POSTPRANDIAL LIPAEMIA

The causal role of an elevated serum cholesterol level in the genesis of atherosclerosis and its clinical sequelae, particularly ischemic heart disease, is now well established. (86) Angiographic studies have also demonstrated a positive relation between the presence of coronary artery disease and serum levels of total cholesterol, LDL cholesterol, and apolipoprotein B. Much of our knowledge about the relationship between lipid and lipoprotein metabolism and development of atherosclerosis and cardiovascular disease is based on measurements in the fasting state. (87) There are several lines of evidence suggesting that postprandial lipemia increases risk of atherogenesis. As far as is known, this is the first study to contribute to the information about postprandial lipaemia in urbanized South Africans with CAD.

Our results show a correlation between postprandial lipemia and the presence of coronary artery disease in urbanized South Africans. The CAD patients with and without metabolic syndrome had similar fasting lipid profiles. However, the fasting TG concentration was the strongest determinant of postprandial lipaemia. Small, dense

LDL particles were present in more than 70% of the patients with CAD. Fasting lipid profiles and postprandial responses to the oral fat load were similar in patients with and without metabolic syndrome.

There was a consistent relationship between increased fasting TG concentrations, elevated postprandial TG and the presence of small, dense LDL particles. Prolonged exposure of the endothelium to the TG-rich atherogenic remnant particles might be the reason why postprandial increases in TG account for greater CAD risk. In a study by Patsch et al (88), on an Austrian cohort, demonstrated the association between late postprandial triglyceride levels and CAD. Is the postprandial lipemia a better surrogate marker of ischemic heart disease than Apo B? The Swedish prospective study AMORIS, found Apo B to be a stronger marker of CVD risk than LDL cholesterol. (89). The INTERHEART study used the ApoB/Apo A-1 ratio. The only constraint for using ApoB is finance especially within the African context. It is very expensive. In the future, more studies are needed to compare the reliability and sensitivity of postprandial lipemia and Apo B.

4.4 EPIDEMIOLOGICAL TRANSITION

An epidemiological transition is now occurring in the developing world: the major causes of death are changing from infectious diseases to non-communicable diseases such as coronary heart disease (see Table 3 below). Urbanization, industrialization and socio-economic development have resulted in significant changes in lifestyles. The WHO has estimated that by 2020, one third of the global burden of disease will be attributed to non-communicable diseases.

Table 3: Epidemiological transition

Yusuf et al Global Burden of Cardiovascular Diseases, Part 1 ***2749***

Modified Model of the Stages of Epidemiologic Transition as it Pertains to Cardiovascular Diseases

Stages of Development	Deaths From CVD, % of Total Deaths	Predominant CVD and Risk Factors	Regional Examples
1 Age of pestilence and famine	5-10	Rheumatic heart disease, infections and nutritional cardiomyopathies	Sub-Saharan Africa, rural India, South America
2 Age of receding pendemics	10-35	As above +hypertensive heart disease and hemorrhagic strokes	China
3 Age of degenerative and man-made diseases	35-65	All forms of stroke, ischemic heart disease at young ages, increasing obesity and diabetes	Urban India, former socialist economies, aboriginal communities
4 Age of delayed degenerative diseases	<50	Stroke and ischemic heart disease at old age	Western Europe, North America Australia, New Zealand
5 Age of health regression and social unheaval	35-55	Re-emergence of deaths from rheumatic heart disease, infections, increased alcoholism and violence; increase in ischemic and hypertensive diseases in the young	Russia

During Stages 1 to 4, life expectance increases, whereas in Stage 5 life expectancy decreases compared with stages 4 and even 3.

(Circulation. 2001;104:2746-2753)

Chronic diseases of lifestyle are a group of diseases that share similar risk factors because of exposure, over many decades, to unhealthy diets, smoking, and a lack of exercise and possibly stress. People are moving from the traditional healthy ways of living and are increasingly adopting unhealthy lifestyle practices. Shifts in dietary intake and physical activity patterns to higher fat intake and inactivity are contributing factors. The globalization of food production and marketing is also contributing to the increasing consumption of energy dense foods poor in dietary fibre and several micronutrients. It has been the historical experience of the developed countries that the cardiovascular the epidemic usually commences in members of the higher social classes - later the risk permeates across the social spectrum.

A school of thought believes epidemiological transition is misplaced in Africa. (90) They are of the view that infectious diseases should be Africa's public health priority. I

disagree. It would be negligent and dangerous to ignore the socio-economic risks posed by cardiovascular diseases. South Africa is not immune to epidemiological transition just like any other developing country. China has experienced an epidemiological transition shifting from the infectious to the chronic diseases. (91) China already has 177 million adults with hypertension; furthermore, 303 million adults smoke, which is a third of the world's total number of smokers.

The Interheart Africa study showed that developing countries are undergoing globalization and epidemiological transition. The data confirms that people from Africa, who are exposed to these known major CVD risk factors, are at risk of developing AMI, as are other people across the globe. Three are only five common risk factors that account for 89.2% of the Acute Myocardial Infarction (AMI), i.e.: current /former tobacco smoking, hypertension, diabetes, abdominal obesity and lipoprotein apoB/apoA1 ratio.

The strong influence also showed that one risk factor is markedly modified by other risk factors. Rapid urbanization of the black population in Soweto places it at risk for several diseases. Soweto, an acronym for South Western Townships, with an estimated population of 1.1 million, has one of the largest urban populations of black Africans on the African continent. It accommodates black people migrating from rural areas.

Rapid transition is expected with an increase in the burden of cardiovascular disease. Many black people in urban South Africa have hypertension related to high salt intake, have adopted diets similar to those in western countries and have changed to a sedentary lifestyle. Even rural black people in South Africa have a CVD risk profile that predisposes them to the emergence of atherosclerosis and related chronic diseases. (92) The ongoing Heart of Soweto study will provide important insights into the prevalence of heart disease in the future.

4.5 CAROTID INTIMA-MEDIA THICKNESS AND CAD

The study findings support the hypothesis, that CIMT is a predictor of coronary artery disease in South African black patients. (93) The study group consisted of patients

admitted with angiographically confirmed coronary artery disease. The majority of patients were on treatment for hypertension. Diabetes and HIV positive patients excluded from the study, due to their high risk of vascular disease.

The thickness of the intima-media complex increases with age, hypertension, diabetes mellitus and hyperlipidaemia. The intima-media thickness was more pronounced in obese patients and those with abnormal fasting glucose levels. Bearing in mind that diabetics were excluded from the study, the effect of high fasting blood glucose levels on the CIMT was significant.

There was a good correlation of CIMT against fasting blood glucose levels of the whole group in simple linear regression analysis ($r= 0.35$; $P=0.03$). In addition, 100% of subjects with high fasting blood glucose had an increased CIMT.

The median percentile scores show a progressive increase as the number of vessels involved increases. The average IMT for the control subjects was lower than in CAD cases. In three vessel diseases, the IMT average was 1.29 mm.

In the study done by Kablak-Ziembicka et al (94), showed that increased CIMT was positively and linearly related to CIMT: subjects with a greater number of vessels involved showed greater increases in CIMT. In addition, they showed that a CIMT over 1.15mm was predictive of a 94% likelihood of having CAD. The study by Geroulakos et al (95), showed that there was a significant positive linear trend between CIMT and the number of involved vessels ($p<0.0001$, $r=0.44$). It demonstrated a high positive predictive value and specificity for the presence of CAD, if a corrected-for-age CIMT >0.85 mm is used as a cut-off point for the prediction of severe CAD.

The findings in the study support previous studies, where increased CIMT was correlated with evidence of angiographically proven CAD. (96) CIMT could be useful as a screening tool in a population with a low prevalence of CAD. (97)

4.6 INFLAMMATION, METABOLIC SYNDROME AND CAD

Recent research has shown that inflammation plays a key role in coronary artery disease and other manifestation of atherosclerosis. (98) Elevated levels of inflammatory markers, particularly C-reactive protein, indicate an increased risk of coronary heart disease (99) the study assessed the integrated relationship between MS, obesity and inflammation in urban South African black patients with CAD, some of whom also had MS. The major findings were that, of the three markers, only leptin was significantly higher in patients with MS compared to patients without MS. Leptin levels in both women and men with MS were significantly higher than in those without MS. The higher leptin levels measured in the patients with MS reflected their greater degree of body fat mass and abdominal obesity compared to those without MS.

Previous studies as well as this study's contribution to a report on body composition, have stated that BMI and WC are important determinants of increased leptin levels. Positive correlations between leptin and hs-CRP, as well as hs-CRP and BMI suggested that these markers were closely related to obesity and inflammation in the CAD patients.

The study was limited by the relatively small groups of patients and control subjects and by the low number of patients who fulfilled all five criteria for the MS. It was concluded that leptin, in addition to being an indicator of obesity, was also a useful marker of inflammation in our CAD patients.

4.7 NUTRITION AND CAD

The study investigated the role of diet, socio-demographics and physical activity in a black South African population with CAD compared to a healthy control group. No differences were found between the dietary intake, physical activity or socio-demographics of the two groups, except for vitamin C intake ($p=0.049$), with the controls having a median vitamin C intake within the DRI range (94mg) and the CAD patients an intake well below (47mg). Compared to dietary guidelines for the prevention of CAD, saturated fatty acid and cholesterol intake were above the recommended intake and fibre, folate and vitamin E were all below the recommended intake for both groups.

In order to determine the role of urbanisation on diet, the study population's diet was compared to that of a traditional rural group and an urban group with a comparable socio-demographic status. The study population's diet found to be westernized compared to both the rural and urban group.

In conclusion, when the confounding factors of age and obesity were accounted for, there were no differences in the diet, physical activity or socio-demographics of the CAD patients and the control group, except for vitamin C intake; both groups had a micro-nutrient intake below the recommended level. While diet is affected by urbanisation, differences in dietary intake were observed between the two urban groups, regardless of the similarity in their socio-demographic profile.

CHAPTER 5: STUDY LIMITATIONS

The relatively small number of patients studied, particularly the low number of women, hampered a wider extrapolation of the study results. The findings should, therefore, be considered as somewhat preliminary, awaiting confirmation in a larger study. The strengths of the study include a detailed assessment of MS risk factors and the exclusion of confounding variables (such as diabetes mellitus and severe hypercholesterolemia) from the patient cohort.

An additional limiting factor was that the criteria for postprandial TG levels, similar to the diagnostic glucose tolerance test, have not been specified for the OFTT. This lack of standardization made it difficult to compare studies because researchers have used different meals containing variable amounts of fat, carbohydrate and protein.

Furthermore, the length of all studies was not uniform. Some studies measured TG concentration every hour; others took measurements every two hours.

This is also the first study on postprandial lipaemia in urbanized South Africans with CAD.

The study also highlighted the clinical relevance of MS, its likely impact on morbidity and mortality and that its identification is, therefore, important in risk assessment of patients with CAD.

CHAPTER 6: GAPS IN OUR KNOWLEDGE

CVD and its major component, heart disease, exists in epidemic proportions in Western, developed countries. The causes and consequences of an epidemic of CVD, in the Western world have been comprehensively documented. In contrast, there is a paucity of data to describe the emergence and impact of CVD in low to middle-income countries.

Historically, the black population was perceived to be immune to coronary artery disease. However, the emergence of a range of modifiable risk factors, including smoking, obesity, hypertension and hyperlipidemia in the context of historically low rates of CAD, has major public health implications. Africa faces the emergence of chronic diseases, including cardiovascular diseases, diabetes and cancers. The overall burden of CVD is predicted to rise by approximately 150% in the developing world within the next 20 years. In Africa alone, it is predicted CVD will affect 1.3 million people per annum during this period.

With industrialization, the major causes of death have shifted from infectious and nutritional deficiencies to disorders that are more chronic: a phenomenon known as epidemiologic transition. It is, therefore, imperative to expedite CVD studies in Africa to improve the understanding of the risk factor profile of this emerging epidemic in the black population. Other institutions will copy the Heart of Soweto cardiovascular disease registry, at CHBH in Soweto.

The paucity of data on CVD is a serious shortcoming that needs to be corrected urgently due to its danger to the economic growth of the country. Anecdotal reports put the cardiovascular disease economic burden at 2% - 3% of gross domestic product (GPD).

CHAPTER 7: FUTURE STUDIES

South Africa is facing a triple burden from infectious diseases, chronic diseases of lifestyle and trauma. Improved socio-economic conditions meant decreased physical activity and an increased intake of saturated fats, smoking and chronic disease of lifestyle. The increasing global burden of CVD needs to be addressed. Intervention to prevent morbidity and mortality from chronic diseases need to be cost effective and financially feasible in countries of low or middle income. (100)

CVD is a major public health challenge, especially for low income and middle-income countries, where 80% of these deaths occur. (101) Treatment is likely to account for an increasing proportion of health-care expenditure in these countries. (102) For successful prevention and control of cardiovascular disease, the present distribution of risk factors be reduced. Financial and time constraints make it difficult to conduct long-term cohort studies. Public health action cannot afford to wait that long to initiate interventions.

Proven cost effective strategies are available for reducing exposure to chronic disease risk factors in low-income and middle –income settings. Modification of risk factors reduces the mortality and morbidity in people with diagnosed and undiagnosed CVD. Effective and inexpensive treatments are under-used in developing countries, even in secondary prevention this despite proven benefits. A combination of individual prevention approach and population level intervention, based on opportunistic screening, identification of high-risk individuals by easily measurable risk factors, and treatment with a multi-drug regimen, could avert some CVD.

The paucity of data on cardiovascular diseases in South Africa, however, limits any effective intervention strategies. Scaling up this intervention is essential for achieving the goal of yearly reduction in rates of chronic disease deaths in the future. The WHO Comparative Risk Assessment project estimated the number of deaths from chronic diseases is averted if the distributions of major risk factors were reduced: worldwide, millions of deaths could be averted if the selected measures to reduce tobacco and salt exposure were implemented.

A shift from the management of single risk factors to total cardiovascular risk prediction and management will enable health resources to be targeted at individuals who are most in need and most likely to benefit.

Financial cost estimates regarding cardiovascular disease in South Africa is required. The impact of HIV/AIDS on cardiovascular diseases needs to be studied further. The expected epidemiological transition in sub-Saharan Africa is being radically altered by the HIV/AIDS pandemic, and the drastically shortened life expectancy in the region means that the age structure is skewed to those who are younger.

Certain areas of CVD epidemiology need special attention in the studies. Nutritional epidemiology in diverse cultural settings with widely varying dietary practices faces the challenge of identifying affordable and culturally acceptable diets for each population. Food labeling is critical in empowering consumers by providing the necessary dietary information. Culturally appropriate dietary studies are needed as part of a patient's education. Cultural beliefs are more important in influencing consumers' food choices.

Political action is needed to reverse the negative trends of obesity and sedentary habits, ranging from fighting against the fast food and sugar industries to safe bicycle paths and healthy school meals. ((103) Health policy research be strengthened. The existing health care infrastructure is equipped to handle mostly the pre-transitional agenda of infectious and nutritional deficiency disorders. Health education is a priority. Public health action for CVD in the developing countries linked to a policy-relevant research agenda. Advancement of global cardiovascular disease prevention needs strong international leadership. (104)

CHAPTER 8: RECOMMENDATIONS

In the book, "Fat Land – how Americans became the fattest people in the world", Greg Critser writes: "…in the twenty-first century, we have put ourselves in the first circle of fat hell. How we get out of that hell depends not upon prayer, but rather upon a new sense of collective will - and individual willpower". It is willpower combined with collective effort from the individual to the state. Successful intervention will require a holistic approach from all major stakeholders, i.e. the policy makers, business sector, health care industry, civil society and different communities.

8.1 SHORT TO MEDIUM TERM

8.1.1 Government

Central government should play an active role in promoting a healthy lifestyle. State officials should be part of CVD awareness campaigns, such as the September Heart Awareness Month. This will go a long way to changing existing perceptions and stereotypes about CVD.

8.1. 2 Schools

A healthy lifestyle should start at pre-school level. Fitness projects such as Health of the Nation, organized by Sporting Chance and Virgin Active, deserve support that is more public. Bring back the physical training period at schools. Lastly, every school should have a dietician.

8.1.3 Dieticians

There is a serious shortage of dieticians in the country. A 3000-bed hospital, such as CHBH, has less than 10 dieticians. Something is wrong here. Some areas in Soweto have never seen a dietician.

A leading Sunday newspaper reported on a study by the University of the Witwatersrand (Sunday Times, 12 October 2008). It found teenagers in Soweto eat more fatty and salty food than children in the USA. This is due to a lack of knowledge and ignorance amongst the youngsters about correct eating habits. Hence, the high levels of overweight and obesity in the townships.

It is in the interests of the country to make the profession more attractive and economically viable.

8.1.4 Media

The BBC's Fat Challenge campaign illustrates the power of the media in changing behaviour. The aim of the Fat Challenge is to encourage citizens to adopt an active lifestyle. South African media can emulate this example by being part of the solution to the obesity epidemic.

8.2 LONG TERM

8.2.1 Health policies for a healthy lifestyle

The ban on junk food adverts at schools tuck shops is a step in the right direction. The amendment of the Foodstuffs, Cosmetics and Disinfectant Act of 1972, is a bold move by the state. The draft regulations include mandatory markings on most foodstuffs and new food labeling. This will allow customers to make informed choices about the food they are buying. More policies of this nature are needed to encourage a healthy lifestyle.

8.2.2 Primary health care

Disease prevention is a primary care responsibility. Currently, the country's clinics are not functioning well. Many patients have no confidence in the services provided. Clinics are under-staffed and demoralized. It is critical that these structures are functioning.

Secondly, there is a need to train more health educators. These foot doctors promote disease prevention. Many unemployed graduates can assist in this task.

CHAPTER 9: CONCLUSION

It is postulated that, by 2025, CVD will overtake HIV/AIDS as the leading cause of death in Africa. The post-apartheid period has witnessed massive economic growth, urbanization, improved sanitation and better access to clean water. Higher disposable income within the black communities has led to increased sedentary lifestyles characterized by a lack of exercise and an increase in smoking and Westernization of diet.

Epidemiological transition is a common feature of the developing economies worldwide. This thesis is the first step in addressing the biochemical genesis of this transition within the black population. This is the first work of this kind in South Africa to study the relationship between epidemiological transition, metabolic risk factors and CAD prevalence within the black population.

In concluding, it is appropriate to recap the main hypotheses and how were these answered.

In papers 1 and 2 it was demonstrated that MS, undiagnosed diabetes mellitus and insulin resistance are highly prevalent in urbanized South Africans with CAD. Obesity-driven diabetes is an important risk factor in CAD.

Papers 3 and 4 showed there is a difference in the biochemical risk factor profile of black subjects with CAD, compared to age matched black controls without CAD. Secondly, there is a postprandial glucose difference in the black CAD subjects compared to age matched controls without CAD. Lastly, there are postprandial lipids differences in lipids and lipoproteins of black CAD subjects compared to age matched controls without CAD.

Paper 5 demonstrated the importance of Carotid intima-media thickness as a surrogate marker of atherosclerosis.

Paper 6, clearly demonstrated that leptin differentiated between CAD patients with and without metabolic syndrome and determined MS status in women and men.

In paper 7, there was not much difference between the results of the CAD cases and control subjects.

In the event of an increasing HIV/AIDS epidemic in sub-Saharan Africa, it is important to fully explore the role of inflammation in the genesis of CAD. More epidemiological studies are needed on the nutritional transition within black communities and the use of CIMT as a screening tool. Government must lead the fight against the emerging CVD threat. Investing in CVD research and promoting intervention studies is the first step in the right direction.

The socio-economic implications posed by CVD cannot be ignored. Action must be taken –NOW! In the words of Olusegun Obesanjo, the former president of Nigeria: "…we cannot afford to say …we must tackle other diseases first - HIV/aids, malaria, tuberculosis – then we will deal with chronic diseases. If we wait even 10 years, we will find that the problem is even larger and more expensive to address."

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CHAPTER 11: APPENDICES

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APPENDIX A

CORONARY ARTERY DISEASE IN THE EMERGING BLACK POPULATION OF SOUTH AFRICA – IS THERE ETHNIC IMMUNITY?

Subject Information and Consent Form (Controls)

I am a doctor working in the Department of Medicine at the Johannesburg Hospital.

You have recently had an angiogram of the arteries supplying your heart. You showed no evidence of obstruction or narrowing in those arteries. In other words you have no evidence of coronary artery disease.

You have a normal or only mildly elevated blood cholesterol level. Yet some people with a similar cholesterol level develop coronary artery disease. My colleagues and I aim to determine whether those who develop coronary artery disease have higher levels of cholesterol and/or glucose in your blood after eating than people who do not have coronary artery disease.

We would like you to participate in this study.

The study involves three tests that will be done on three separate occasions.

Test 1. Oral glucose Tolerance Test

You will be asked to fast, only to drink water from 20h00 on the night before the test. At 8h00 the next morning (day of the test) we will take some blood samples and then ask you to drink 75gm. (a teacup) of glucose (sugar). Thereafter we will take a few tubes of blood from you at half hourly intervals for 2 hours. This test will be completed by 10h00. The total amount of blood that will be taken will be approximately 80 ml (1/3 cup). We will also ask you to provide us with a urine sample.

Test 2. Oral Fat Tolerance Test

You will be asked to fast, only to drink water, from 20h00 on the night before the test. At 08h00 you will be asked to drink 350mls chocolate or strawberry flavoured fat drink (the drink is made up of cream, chocolate or strawberry flavoured syrup, sugar and powdered milk). Thereafter we will take a few tubes of blood from you at two hourly intervals up until 16h00. The total amount of blood that will be taken will be approximately 70 ml (1/3 cup). We will also ask you to provide us with another urine sample.

Test 3. Euglycaemic Clamp

This is the final test. You will again be asked to fast. A small cannula (needle) will be inserted into a vein in your arm and a small amount of insulin and glucose will be run into your vein. Blood samples will be taken frequently from the cannula (needle) in your arm. This test aims to assess how sensitive you are to insulin and will last approximately three hours. The total amount of blood that will be taken will be approximately 50 ml (¼ cup). We will also ask you to provide us with another urine sample.

The total amount of blood from all three tests combined will be approximately 200 ml (less than 1 cup).

You will be constantly supervised by medical personnel during all the above tests. All three procedures are safe and you are unlikely to come to any harm. There may however be some pain and discomfort at the site at which we take the blood samples. Nausea may be experienced after swallowing the glucose (sugar) or fat meal, but this also is unlikely.

You will also be questioned by a dietician about your regular diet and will be questioned about your level of physical activity. This will take approximately 30 minutes.

From this study we hope to understand better why people with average fasting cholesterol levels can still get coronary artery disease. With a better understanding of the cause, we will hopefully be able to prevent coronary artery disease more effectively in the future.

Participation in this study is voluntary and you are free to refuse to participate or to withdraw your consent at any time. Such refusal will not affect your regular treatments or medical care in any way. In the study, you will be identified by a study number only. Your results will therefore remain confidential and will not be disclosed to anyone without your approval.

If you have any questions or concerns about the study at any time you can contact either Srs. Nancy Holden or Nomsa Ramela in the Department of Chemical Pathology Day Ward at 489-8495 or Doctor Lucas Ntyintyane at 488-3818 or Professor Derick Raal at 488-3538.

I have fully explained the procedures and have explained the purposes of the study. I have asked whether or not any questions have arisen regarding the procedure and have answered these questions to the best of my ability.

Date: …… Doctor: …….

I have been fully informed as to the procedures to be followed, and have been given description of the attendant discomforts, risks and benefits to be expected. In signing this consent form I agree to participate in the study and understand that I am free to refuse to participate or to withdraw my consent at any time. I understand also that if I have any questions at any time, they will be answered.

Date: …… Subject: …….

SUBJECT QUESTIONNAIRE

Subject number		Hospital No	
Name		Contact No:	
Date			
Gender			
Date of birth		Age	

First Language
Second language

CASE		CONTROL	

Date of coronary angiography:
Findings:

What is your marital status?	Never married	1
	Married	2
	Divorced	3
	Widower	4

What is your highest qualification?	None	1
	< Std. 6	2
	Std. 6-8	3
	Std. 6-8 + trade	4
	Std. 9-10	5
	Std. 9-10 + trade	6
	Std. 9-10 + academic	7

What is your occupation?	

Do you have a job at the moment?	Yes	1
	No	2
If yes – what kind of job?		
On which days of the week do you work?	Irregular (piece work)	1
	Part time (1-4 days)	2
	Full time (5-6 days)	3

How much money do you earn per month?	R0-100	1
Is it between	R101-500	2

	R501-1000	3
	R1000-2000	4
	R2000-3000	5
	R3000+	6

Do you receive any additional pensions?	Yes	1
	No	2

How much pension do you receive per month?		
Interviewer - Re-evaluate final income category	R0-100	1
	R101-500	2
	R501-1000	3
	R1000-2000	4
	R2000-3000	5
	R3000+	6

Does anybody else contribute money to your household?	Yes	1
	No	2
If yes, how much?		

Does anybody else contribute other resources e.g. food, to your household?	Yes	1
	No	2
If yes, describe.		

How many people eat in your house?			
Children			1
Adults			2
What type of house do you live in?		Traditional	1
		Mokuku	2
		Brick house	3
		Other	4
If other, specify			

Where do you get your drinking water from?	Fountain, river	1
	Communal tap	2
	Tap on premises	3
	Tap in house	4
	Other	5
If other specify		
Do you have access to electricity inside your house?	Yes	1
	No	2

What type of stove do you have?	None	1
	Coal/wood	2
	Gas or paraffin	3
	Electric	4

What type of fridge do you have?	None	1
	Paraffin	2
	Gas	3
	Electric	4

How long have you been living here? (years)	

Where did you live before coming here?	Rural area	1
	Farm	2
	Squatter camp	3
	Township	4

RISK FACTORS FOR CORONARY ARTERY DISEASE

Family history of CAD	YES	NO
In whom		

Family history of elevated cholesterol	YES	NO
In whom		

Do you snuff?	Yes	1
	No	2
Do you smoke?	Yes	1
	No	2
If no – have you smoked regularly before?	Yes	1
	No	2
If yes – what do you smoke?	Cigarettes	1
	Tobacco/pipe	2
	Snuff	3
	Other	4
If other, please describe		
If cigarettes, how many cigarettes do you smoke?	Per day	
	Per week	
If tobacco, how many packages?	Per day	
	Per week	
If snuff, how many parcels?	per day	
	per week	
If other, describe frequency		

How long have you been smoking (years)?	
Interviewer: Calculate pack years	

HYPERTENSION	YES	NO

DIABETES MELLITUS	YES	NO

OTHER:

LIPID LOWERING MEDICATION:
When stopped:
ASPIRIN:
When stopped
OTHER PRESENT MEDICATION:

VASCULAR HISTORY

ANGINA OR MI	YES Date:	NO

CABG OR ANGIOPLASTY	YES Date:	NO

CVA or PVD	YES Date:	NO

Dietary Assessment

Date done:…….

By whom:……..

Examination

Height: …… metres

Weight: …….. Kg

BMI (ht/wt^2):……..…

Blood pressure:…….…… mmHg

Waist circumference:……..….. cm

Hip circumference:…….….. cm

Abdominal circumference:……... cm

Arcus cornelias	Yes	No
Xanthelasma	Yes	No
Thickened Tendo-achilles	Yes	No

Carotid IMT:
Left:……..……..

Right: …….

Plaque: ……...…

Flow: …….

Pulses/bruits:……..…
……..
Cor:……..…
……..……
Other:……..…..
……..…
……..……...
…….……

STATEMENT OF ORIGINALITY

1) STATEMENT OF ORIGINALITY : Papers 1, 2, 3, 4, 6

NAME	RESPONSIBLITY
Lucas Ntyintyane, University of the Witwatersrand	PRINCIPAL AUTHOR IN THE THESIS. INVOLVED IN PATIENT RECRUITMENT, DATA COLLECTION, MANUSCRIPT WRITING AND REVIEW.
Professor Frederick Raal, University of the Witwatersrand	PRINCIPAL SUPERVISOR IN THE THESIS. INVOLVED IN ALL ASPECTS OF THE THESIS, FROM INTELLECTUAL TO FINANCIAL INPUT.
Vanessa Panz, University of the Witwatersrand	WRITING AND EDITING OF MANUSCRIPTS.
Professor Geoff Gill, Liverpool School of Tropical Medicine, UK	STUDY DESIGN, MANUSCRIPT REVIEW AND FINANCIAL INPUT.
CANDIDATE : I declare that this work is wholly my own, except where acknowledged as being the work of others (as listed above). I also acknowledge the contribution of others (as listed above) to this work in STATEMENT OF ORIGINALITY. Dr Lucas Ntyintyane.	PRINCIPAL ADVISOR : I hereby certify that all co-authors have provided their consent for the inclusion of the manuscripts in the thesis and that the co-authors accept the candidate's contribution to the paper as described in this STATEMENT OF ORIGINALITY. Professor Frederick RAAL

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Zaiboonnisa Holland	STUDY SONOGRAPHER
Professor Geoff Gill, Liverpool School of Tropical Medicine, UK	STUDY DESIGN, MANUSCRIPT REVIEW AND FINANCIAL INPUT.
CANDIDATE : I declare that this work is wholly my own except were acknowledged as being the work of others (as listed above) . I also acknowledge the contribution of others (as listed above) to this work in STATEMENT OF ORIGINALITY, Dr Lucas Ntyintyane	PRINCIPAL ADVISOR : I hereby certify that all co-authors have provided their consent for the inclusion of the manuscripts in the thesis and that the co-authors accept the candidate's contribution to the paper as described in this STATEMENT OF ORIGINALITY, Professor Frederick RAAL

3) STATEMENT OF ORIGINALITY: Paper 7

NAME	RESPONSIBLITY
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Professor Frederick Raal, University of the Witwatersrand	PRINCIPAL SUPERVISOR IN THE THESIS. INVOLVED IN ALL ASPECTS OF THE THESIS, FROM INTELLECTUAL TO FINANCIAL INPUT.
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Professor J.C. Jerling, North West University, School of Nutrition	MANUSCRIPT REVIEW AND FINANCIAL INPUT.
Marlien Pieters, North West University, School of Nutrition.	WRITING AND MANUSCRIPT EDITING.
CANDIDATE : I declare that this work is wholly my own except were acknowledged as being the work of others (as listed above) . I also acknowledge the contribution of others (as listed above) to this work in STATEMENT OF ORIGINALITY, Dr Lucas Ntyintyane.	PRINCIPAL ADVISOR : I hereby certify that all co-authors have provided their consent for the inclusion of the manuscripts in the thesis and that the co-authors accept the candidate's contribution to the paper as described in this STATEMENT OF ORIGINALITY, Professor Frederick RAAL.

APPENDIX B

ETHICS APPROVAL

See two pages attached.

APPENDIX C

MANUSCRIPT: THE METABOLIC SYNDROME USING THE NATIONAL CHOLESTEROL EDUCATION PROGRAM AND INTERNATIONAL DIABETES FEDERATION DEFINITIONS AMONG URBANIZED BLACK CORONARY ARTERY DISEASE

See six pages attached.

APPENDIX D

MANUSCRIPT: METABOLIC SYNDROME, UNDIAGNOSED DIABETES MELLITUS AND INSULIN RESISTANCE ARE HIGHLY PREVALENT IN URBANISED SOUTH AFRICAN BLACKS WITH CORONARY ARTERY DISEASE

See six pages attached.

APPENDIX E

MANUSCRIPT: POSTPRANDIAL LIPAEMIA, METABOLIC SYNDROME AND LDL PARTICLE SIZE IN URBANISED SOUTH AFRICAN BLACKS WITH AND WITHOUT CORONARY ARTERY DISEASE

See nine pages attached.

APPENDIX F

MANUSCRIPT: POSTPRANDIAL HYPERGLYCAEMIA IN URBANISED SOUTH AFRICAN BLACKS WITH AND WITHOUT CORONARY ARTERY DISEASE

See eight pages attached.

APPENDIX G

MANUSCRIPT: CAROTID INTIMA-MEDIA THICKNESS IS A PREDICTOR OF CORONARY ARTERY DISEASE IN SOUTH AFRICAN BLACK PATIENTS

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Word Count: 2,633

The Manuscript contains 1 table and 1 figure

Key Words: carotid intima- media thickness, coronary artery disease, atherosclerosis

ABSTRACT

Background - Several studies have shown that increased carotid intima media thickness (CIMT) confers risk of future coronary artery disease (CAD) and stroke. The present

study aimed at investigating whether CIMT is a predictor of CAD in South African Black patients.

Methods and Results - This was a prospective study of 53 patients, 41 men and 12 women, with ages ranging from 30 to 70 years. All patients had undergone coronary angiography for suspected CAD. B-mode ultrasound measurement of the carotid intima-media thickness (CIMT) was carried in all patients, the operator being blinded as to the coronary angiography findings. Twenty-nine of the 38 (76 %) subjects with established CAD had increased CIMT, with an average mean CIMT of 1.13mm. Single vessel disease was present in 12 people, double -vessel disease in 11 people and triple - vessel disease in 12 people. There was a significant positive linear trend between CIMT and the number of involved coronary vessels ($p<0.0001$, $r=0.44$).

Conclusions – Increased CIMT correlated with evidence of angiographically proven CAD. The median percentile scores show a progressive increase as the number of vessel involvement increased. CIMT could be useful as a screening tool, for the presence of CAD, in the South African Black population.

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INTRODUCTION

Urbanisation, industrialisation and socio-economic development have resulted in significant changes in lifestyles globally particularly in developing countries. (1) An epidemiological transition is now occurring in the developing world where the major causes of death are changing from infections to non-communicable diseases of lifestyle resulting in an increased prevalence of cardiovascular disease such as coronary heart disease. (2) The South African National Burden of Disease study for the year 2000

estimated that 17 % of all deaths were due to cardiovascular diseases. (3) Early detection of coronary artery disease (CAD) may well prove to be instrumental in introducing effective treatment and may contribute to reducing mortality. (4) Thickening of the intima-media is commonly recognised as the initial stage in the development of atherosclerosis. (5,6) Several studies have shown that increased carotid intima media thickness (CIMT) confers risk of future coronary heart disease and stroke. (7) The present study aimed at investigating whether CIMT is a predictor of CAD in South African Black patients.

METHODOLOGY

This was a prospective study of 53 patients, 41 men and 12 women, with ages ranging from 30 to 70 years. All patients had undergone coronary angiography for suspected CAD.

Ultrasound Technique

B-mode ultrasound measurement of the carotid intima-media thickness was carried out on all patients as a blind study, the operator being unaware of the coronary angiography findings. A standardised ultrasound technique was used, using a Toshiba System: Nemio Model SSA-550A. The transducer frequency was set at 11MHz for all patients.

Measurements of the intima-media thickness was carried out at the optimum angle of interrogation (OAI), which allowed visualization of the flow tip divider, the common carotid artery (CCA), external carotid artery (ECA) and the internal carotid artery (ICA) from a single selected angle of the carotid arteries at the bifurcation. Doppler was used to verify the identification of the ECA and ICA.

The carotid intima-medial thickness (CIMT) was measured when the two echogenic lines, representing the lumen-intima interface and the media-adventitia interface, were visualized over a length of ≥1cm. Measurements of the CIMT were done manually, using the calliper markers of the ultrasound unit.

The CIMT at the optimal angle of interrogation was measured as the area of maximum thickness at the near and far walls of the CCA, BIF and ICA bilaterally (a total of 12 sites). In cases where calcified plaque obscured the IMT in the bulb, one wall was measured; the thickest measurement in each segment was imaged and recorded as the final measurement. The mean maximum IMT was recorded as the CIMT. Images were stored on a magnetic optical disc as well as on thermal paper.

The CIMT was calculated as the average for 12 sites for all subjects, using the Excel programme for Windows XP. A mean value for the CIMT of > 0.8mm was classified as increased thickness. (8)

Subjects with a previous myocardial infarction had to be at least 3 months post-infarction before recruitment. As the study was designed to assess the effects of cardiovascular risk factors of lifestyle (hypertension, dyslipidaemia, smoking and obesity), subjects were excluded from the study if they were previously diagnosed with diabetes mellitus (DM) or genetic dyslipidaemia, or were positive for HIV infection. Subjects with overt renal, thyroid or liver disease were also excluded. Other exclusions were related to technical considerations which did not allow for measurement of the CIMT, including poor imaging with limited boundary visualization or when there were anatomical constraints, either a high carotid artery bifurcation or a short thick neck and where more than 2 segments were not visualized.

Statistical Methods

The Excel programme for Windows XP was used to calculate the mean maximum IMT (CIMT) of the carotid arteries. To assess the independent value of the CIMT as a predictor of CAD, linear and multiple regression analyses were done, using the GB – STAT for Windows, Version 10, and Dynamic Microsystems Inc. 2004.

To find how IMT behaves as a predictor of CAD severity in the presence of other important cardiovascular disease risk factors we included age, hypertension (> 140/90 mm Hg or if the subject was on anti-hypertension medication), abnormal glucose tolerance, central obesity as assessed by abdominal circumference, dyslipidaemia and cigarette smoking.

RESULTS

Among the 53 participants, coronary angiography results showed that 15 people had no evidence of CAD. This constitutes the Control group. Of the 15 subjects in the control group, ten were males and five females, a ratio of 3 to 1. The mean age was 47 years, with an age-range of 30-65 years. Although there was no evidence of CAD on angiography, eight of the 15 (53%) had increased CIMT. The mean CIMT was calculated as 0.998 mm. Four subjects had hypertension as the only risk factor for CAD, and the CIMT values of this group had a median CIMT percentile value (1.22 mm), which was as great as that of subjects with multiple risk factors.

Characteristics of the study subjects (patients with evidence of coronary artery disease on coronary angiography).

The Study group consisted of the 38 subjects with evidence of CAD on coronary angiography, defined as lesions greater than 50% in one or more of the major coronary vessels (TABLE 1). Single vessel disease was present in 12 people, double -vessel disease in 11 people and triple - vessel disease in 12 people. Coronary angiography results were not available for 3 study subjects. In the study population 29 of the 38 subjects (76 %) had increased CIMT - with a mean CIMT of 1.13mm. The mean age for the Study group was 55.4 years, with ages ranging from 36 to 69 years. Linear regression analysis for CIMT against age shows that increasing age is related to increase CIMT ($r = .223$, $p = 0.17$). Hypertension, defined as a blood pressure of > 140/90 mmHg or if the subject was on antihypertensive medication, was very prevalent, 95 % of the study subjects being hypertensive. Obesity, particularly abdominal obesity (defined as an abdominal circumference of > 92 cm in males and > 88 cm in females) was also highly prevalent, 79 % of the study subjects having increased abdominal circumference. Smoking was also more prevalent in the study group, 62.5 % of the subjects being current or ex-smokers.

Although subjects with diabetes were excluded from the study, 55 % were found to have abnormal glucose tolerance. All the patients with a raised fasting glucose had increased CIMT (100%). There was a good correlation of CIMT with fasting blood

glucose levels of the whole group using simple linear regression analysis (r = 0.35; P=0.03). In addition, 100% of subjects with high fasting blood glucose had an increased CIMT.

Of the patients who were found to have impaired glucose metabolism on glucose tolerance tests, 76.2% had increased CIMT. However, those with normal glucose metabolism (neither raised fasting blood glucose nor an abnormal glucose tolerance test) had a similar percentage of subjects with increased CIMT, 76.4%. 19 patients (5 %) had raised fasting triglyceride (TG) levels of > 1.7 mmol/l, of which 78.95% had increased CIMT. However the percentage with increased CIMT was similar in the 19 subjects with normal triglyceride levels (73.68%). Subjects with high LDL-C levels (LDL-C > 3 mmol/l) had increased CIMT in 93.75 % of cases. LDL-C levels also showed a strong positive correlation with CIMT.

Correlation with extent of coronary vessel involvement

The median percentile scores showed a progressive increase as the number of vessel involvement increases. The median CIMT average was 0.88 mm on those with a single vessel disease, 1.08 mm on those with double vessel diseases and 1.19 mm on those with triple vessel disease (FIGURE 1). There was a significant positive linear trend between CIMT and the number of involved vessels ($r = 0.44$, $p < 0.0001$).

DISCUSSION

The study findings support the hypothesis that CIMT is a predictor of the presence and extent of CAD in South African black patients. (9) Predictors of CIMT included several of the classical CAD risk factors namely age, hypertension, dyslipidaemia, abdominal obesity, and smoking and glucose intolerance. The impact of these risk factors on CIMT has been previously well described. (10, 11) On univariate analysis the key risk factors associated with increased CIMT were hypertension and raised fasting glucose. (12)

Bearing in mind that diabetics were excluded from the study, the effect of high fasting blood glucose levels on the CIMT was significant. Thus in this study, high fasting

glucose levels, which is also an important component of the metabolic syndrome, was an important factor associated with increased CIMT.

The median percentile scores of CIMT show a progressive increase as the number of coronary vessel involvement increased. The findings in the study support previous studies (13, 14), where increased CIMT was correlated with evidence of angiographically proven CAD. The study by Kablak-Ziembicka et al showed that increased CIMT was positively and linearly related to CIMT, subjects with a greater number of vessel involvement showed greater increases in CIMT. In addition their study showed that a CIMT over 1.15mm was predictive of a 94% likelihood of having CAD. The study by Geroulakos et al (15) also showed a significant positive linear trend between CIMT and the number of involved vessels. The study also demonstrated a high positive predictive value and specificity for the presence of CAD if a CIMT >0.85 mm correlated for age, is used as a cut-off point for the prediction of CAD. (16)

Table 1: Clinical characteristics of the studied patients with CAD and without CAD on coronary angiography.

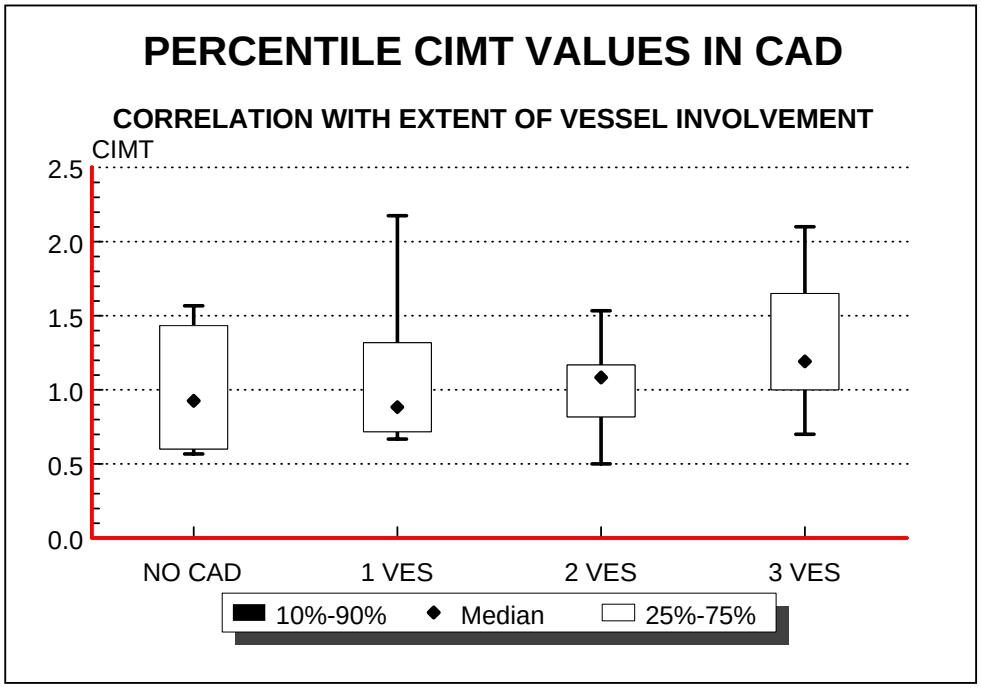
Table 1 Clinical Characteristics of the Studied Patients

	No CAD (n = 15) %	**CAD (n =38) %**
Hypertension (Blood pressure > 140/90 mmHg or on antihypertensive therapy)	**60**	**97**
Increased Abdominal Circumference (male > 92 cm , females > 88 cm)	**33.3**	**79.1**
Abnormal Glucose Tolerance	**0**	**55.26**
Raised Triglycerides (> 1.7 mmol/l)	**26.7**	**50**
Low HDL Cholesterol (men <1.0 mmol/l, women < 1.3mmol/l)	**33.33**	**42.10**
Raised LDL Cholesterol (> 3 mmol/l)	**13.33**	**42.10**
Cigarette Smoking / Previous Smokers	**20**	**62.5**
Mean CIMT(mm)	**0.998**	**1.13**
% with increased CIMT (> 0.8 mm)	**53**	**76**

The limitations are inherent in the study design, which recruited subjects undergoing coronary angiography for a suspected clinical diagnosis of CAD. The study population, a total of 53 subjects, was thus highly selected, without the possibility of case matching, particularly for the dominant risk factor, hypertension, which was present in every study

subject but one. (17) Future researchers will be faced with the challenge of recruiting a larger group of participants to allow for a controlled study within this population with a rising prevalence of CAD. Better definition of the impact of each of the risk factors on CIMT and consequently CAD should be the role of future studies.

FIGURE 1. CIMT WITH EXTENT OF VESSEL INVOLVEMENT



The above graph indicates a correlation of the CIMT and the extent of CAD

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APPENDIX H

MANUSCRIPT: LEPTIN, ADIPONECTIN AND HS-CRP IN RELATION TO THE METABOLIC SYNDROME IN URBAN SOUTH AFRICAN BLACKS WITH AND WITHOUT CORONARY ARTERY DISEASE

Leptin, Adiponectin and hs-CRP in Relation to the Metabolic Syndrome in Urban South African Blacks with and without Coronary Artery Disease

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Abstract

Background: Metabolic syndrome (MetS) and coronary artery disease (CAD) are increasing in urban black South Africans during their transition from a rural to a western lifestyle. Inflammation is frequently associated with MetS and CAD. This study evaluated markers of inflammation in black CAD patients, some of whom had MetS.

Methods: MetS was defined according to International Diabetes Federation criteria. Inflammatory markers leptin, adiponectin and high sensitivity C-reactive protein (hs-CRP) were measured in 40 patients and 20 control subjects.

Results: MetS was present in 23 patients and absent in 17 patients. Leptin was the only significantly higher marker in patients with MetS compared to patients without MetS ($p < 0.01$). Leptin was higher in women than men ($p < 0.01$), and higher in both genders with MetS ($p < 0.03$ and $p < 0.04$, respectively). Leptin levels rose significantly with increasing MetS criteria ($p < 0.05$). hs-CRP concentrations were elevated in both patient groups. Positive correlations were found between leptin and body mass index (BMI) ($r = 0.7107$; $p < 0.0001$), waist circumference (WC) ($r = 0.4981$; $p < 0.002$), and hs-CRP ($r = 0.3886$; $p <0.02$).

Conclusions: Leptin differentiated between CAD patients with and without MetS, and determined MetS status in women and men. Leptin was the only marker that increased with additional MetS criteria. Elevated hs-CRP concentrations indicated an inflammatory state in CAD patients. Association of leptin with BMI, WC and hs-CRP revealed a close link with MetS, obesity and inflammation in urban black South African CAD patients.

Introduction

The metabolic syndrome (MetS) is widely used nowadays to describe a cluster of metabolic abnormalities that predisposes an individual to cardiovascular disease and diabetes mellitus (DM).[1] During the past two decades, there has been a striking increase in the prevalence of MetS in parallel with the global epidemic of obesity. Numerous

researchers have found varying prevalence rates in populations from diverse ethnic backgrounds ranging from the Japanese (11%),[2] Arabs (16%),[3] Asian Indians (35%)[4] to black (34%) and white (39%) Americans.[5] An even higher rate (58%) was reported by us in South African Blacks with coronary artery disease (CAD).[6]

Thirty years ago, CAD was a rarity in the black population of South Africa.[7] In recent times, however, accumulating evidence shows that CAD is emerging rapidly in this group as they migrate from rural to urban areas and adopt a western lifestyle of less physical activity and a diet high in refined foods.[8,9] New data from the Heart of Soweto study of a large urbanized black community and our own research has found a high prevalence of risk factors, namely, obesity, hypertension, dyslipidemia and hyperglycemia.[10,11,12] These multiple risk factors that relate to CAD are also documented criteria for MetS.[1] Other population-based studies by Lakka *et al.* [13] and Isomaa *et al.*[14] of the relationship between CAD and MetS have demonstrated that patients with the syndrome had a two- to four-fold increased risk of mortality. Therefore, identifying MetS is clinically important mainly because of its association with CAD.

There is growing support for the concept that inflammation is involved in the initiation and progression of CAD[15, 16, 17] and a proinflammatory state is considered to be a characteristic feature of MetS.[18] It is acknowledged that obesity, particularly abdominal obesity, which is the primary diagnostic criterion for MetS, is also accompanied by systemic low-grade inflammation.[19] However, the link between CAD, MetS, obesity and inflammation is not yet fully understood. One postulated mechanism is that adipocytes secrete increased levels of cytokines into the circulation which may induce an inflammatory response throughout the body.[20] "Adipokines"—cytokines of the adipose tissue, including leptin and adiponectin, in turn, stimulate the production by the liver of large amounts of acute phase reactants such as C-reactive protein (CRP).[20] This inflammatory process in atherosclerotic arteries may lead to elevated blood levels of these inflammatory markers in patients with CAD.[17] Therefore, the value of measuring them in CAD patients lies in the ability of these markers to detect inflammation and potentially identify the presence of MetS.

Leptin, from the Greek *leptos* for thin, is probably the best characterized of the inflammatory markers. It is involved in the regulation of appetite and energy homeostasis, and leptin levels are elevated with increased adiposity[21]. Adiponectin is distinct from the other adipokines in that it is inversely related to obesity.[22] CRP, which was originally discovered as a protein that reacted with the C-polysaccharide of Pneumococcus, rises dramatically during the acute phase of the inflammatory cascade[23]. A high sensitivity CRP (hs-CRP) assay designed for greater accuracy in measuring the lower concentrations of CRP at the early onset of inflammation is used currently for cardiovascular risk assessment. At this stage of black South Africans' epidemiologic transition, relatively little is known about leptin, adiponectin and hs-CRP in this population. The aim of this study was to evaluate these markers in urban black patients with CAD, some of whom also had MetS.

Methods

Patients and control subjects

Forty patients (33 men, 7 women) and 20 control subjects (13 men, 7 women) who attended the Chris Hani Baragwanath Hospital in Soweto, one of the largest hospitals in the southern hemisphere, participated in the study. Patients and control subjects were classified according to the number and extent of lesions in coronary arteries, which were verified by a coronary angiogram performed in the preceding 24 months. All of the patients had significant CAD, which was defined as greater than 50% lesions in one or more major coronary arteries. None of the control subjects had evidence of coronary atherosclerosis on coronary angiography. Patients who had a previous myocardial infarction (MI) were at least three months post-MI before the study started. The majority of patients (95%) and control subjects (75%) had moderate hypertension (blood pressure $\geq$ 130/85 mmHg) and were taking antihypertensive medication.

Patients who had a dominant risk factor such as severe hypercholesterolemia or DM, which had been previously diagnosed by a physician, and were using medication, were excluded. Other exclusion criteria were: human immunodeficiency virus (HIV)-positive status; overt liver, renal or thyroid disease; and smoking more than 20 cigarettes per day. All participants gave written informed consent to take part in the study, which was

approved by the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg, South Africa.

Definition of the metabolic syndrome

MetS was defined according to International Diabetes Federation (IDF) criteria[1], which require waist circumference (WC) ≥ 94 cm in men or ≥ 80 cm in women *plus* any two or more of the following four risk factors: (1) triglycerides (TG) ≥ 1.7 mmol/L; (2) high-density lipoprotein cholesterol (HDL-C) < 1.03 mmol/L in men or < 1.29 mmol/L in women; (3) systolic blood pressure (BP) ≥ 130 mmHg, diastolic BP ≥ 85 mmHg, or on treatment ; and (4) fasting plasma glucose ≥ 5.6 mmol/L or previously diagnosed DM. Because the definition does not specify WC measurements for black Africans, European WC measurements were applied in this study.

Design

Four weeks before the study started, lipid-lowering medications such as statins and fibrates were discontinued. The night before the test, participants were asked to refrain from smoking and fast for 12 hours. On the day of the study, each participant underwent a clinical examination that included an interview and a venipuncture. Height, weight, WC and hip size were measured, and body mass index (BMI) and waist-to-hip ratio (WHR) were calculated. Fasting blood samples were analyzed for triglycerides (TG), high density lipoprotein cholesterol (HDL-C), glucose, leptin, adiponectin and hs-CRP. Questionnaires were used to obtain information on demographic variables, medical history, medication use, dietary habits, physical activity and smoking status.

Laboratory assays

Concentrations of TG, HDL-C, and glucose were determined by enzymatic colorimetric methods using a Hitachi automated analyzer and reagents were supplied by Roche Diagnostics GmbH (Mannheim, Germany) (reference ranges: TG 0.5—1.5mmol/L; HDL-C > 1.2 mmol/L; glucose 3.9—5.5 mmol/L). Leptin and adiponectin levels were measured by enzyme-linked immunosorbent assays (ELISA) (R&D Systems Inc., Minneapolis, MN) (reference ranges: leptin 0.5—12.7 ng/ml in men and 3.9—30.0

ng/ml in women; adiponectin 4.0—20.0 μg/L in men and 5.0—28.0 μg/L in women). An immunoturbidimetric assay was used to measure hs-CRP (Tina-quant®, Roche Diagnostics GmbH, Mannheim, Germany) (reference intervals: low risk < 1.0 mg/L, average risk 1.0—3.0 mg/L, high risk > 3.0 mg/L).

Statistical Analysis

Data were analyzed using the GB-STAT program (Dynamic Microsystems, Inc., Silver Spring, MD) with a p value < 0.05 considered significant. Analysis of variance (ANOVA) was used to compare more than two groups. Continuous variables were compared by the *t*-test, Mann-Whitney *U*-test, or Wilcoxon Signed-Rank test for parametric or non-parametric data, respectively. Results are expressed as mean ± standard deviation (SD) or as proportion (%). Linear regression analysis was performed to reveal any significant correlations.

Results

Classification of the metabolic syndrome

Measurements of WC and the number of other criteria for MetS were assessed according to the IDF definition. This allowed classification of the 40 patients into two groups. MetS was present in 23 patients and absent in 17 patients. Three of the control subjects had MetS as defined, but they had no evidence of CAD on angiography and, therefore, were included in the study.

Fasting profiles of the study groups

Table 1 summarizes the mean anthropometric measurements and fasting biochemical variables of all the CAD patients, CAD patients with and without MetS, and control subjects. Patients and control subjects were matched for age, WHR, BMI and WC, all p > 0.5. BMI was significantly higher in patients with MetS compared to those without MetS (p < 0.0001) and lower in patients without MetS than control subjects (p < 0.02). As expected, WC, which is the main diagnostic criterion for MetS, was significantly

higher in patients with MetS than patients without MetS ($p < 0.0001$) and control subjects ($p < 0.01$).

Systolic and diastolic BP readings were highest in patients with MetS. Most of the patients with MetS (96%) and without MetS (94%) had moderate hypertension and were taking antihypertensive medication. Patients with MetS had a slightly elevated TG level and a slightly lower HDL-C concentration than patients without MetS and control subjects. Glucose concentrations in both MetS patient groups were higher than control subjects, significantly so in patients with MetS ($p < 0.01$).

Leptin levels were significantly higher in patients with MetS than patients without MetS ($p < 0.01$) and also higher in control subjects than patients without MetS ($p < 0.02$). Adiponectin level in patients with MetS was slightly lower than in the other groups. Concentration of hs-CRP was slightly but not significantly higher in patients with MetS, and both groups of patients were higher than control subjects, significantly so in patients with MetS ($p < 0.01$).

Marker measurements grouped by gender and MetS status

Table 2 presents the mean marker measurements grouped by gender in patients with and without MetS. Leptin was significantly higher in women than men ($p < 0.01$). Leptin levels in women and men with MetS were significantly higher than women and men without MetS ($p < 0.03$ and $p < 0.04$, respectively). There were no substantial differences between women and men in either adiponectin or hs-CRP concentrations.

Marker measurements grouped by number of MetS criteria

Table 3 shows the mean marker measurements grouped by the number of criteria for MetS. When the markers were analyzed according to increasing MetS criteria, only leptin showed a statistically significant difference in levels as the number of MetS criteria increased ($p < 0.05$). Leptin levels increased progressively, with those patients who had the most criteria having the highest level. In general, concentrations of adiponectin decreased and hs-CRP increased with additional criteria, but values did not change in a linear manner and the differences were not significant.

Linear regression analysis

Linear regression analysis of the total patient group ($n = 40$) in which leptin, adiponectin, and hs-CRP were the dependent variables and age, WHR, BMI, WC, systolic and diastolic BP, TG, HDL-C, and glucose were the independent variables, revealed that the highest positive correlations were between leptin and BMI ($r = 0.7107$; $p < 0.0001$), leptin and WC ($r = 0.4981$; $p < 0.002$), and leptin and hs-CRP ($r = 0.3886$; $p < 0.02$) (Fig. 1). Leptin also correlated with adiponectin albeit in the opposite direction ($r = -0.3411$; $p < 0.04$), whereas hs-CRP correlated positively with BMI ($r = 0.3400$; $p < 0.04$).

Discussion

This study assessed the integrated relationship between MetS, obesity and inflammation in urban South African black patients with CAD, some of whom also had MetS. The main findings were that, of the three markers, only leptin was significantly higher in patients with MetS compared to patients without MetS. Leptin levels in both women and men with MetS were significantly higher than in those without MetS. When the markers were analyzed according to increasing MetS criteria, leptin alone rose significantly with more criteria. We also found that concentrations of hs-CRP were elevated both in CAD patients with and without MetS. In the total patient group, leptin correlated significantly with indicators of obesity (BMI, WC) and inflammation (hs-CRP).

The higher leptin levels measured in our patients with MetS reflected their greater degree of body fat mass and abdominal obesity compared to those without MetS. Recognized gender-specific differences documented in the literature[24] were also noted as leptin levels in our patients were higher in women than men. The significant increase in leptin levels when additional MetS criteria were present was an interesting finding that has not been observed before in black South African patients. However, the inclusion in our study of patients with established CAD implies that most of them would have had more than one of the five risk factors that are common to both CAD and MetS. Indeed, our earlier research has shown that increased WC, together with

hypertension and elevated glucose concentration comprised the most frequent combination (20.8%) in our MetS patients.[25] The inverse relationship between obesity and adiponectin is well known[22] and because our patients with MetS had greater WC and BMI, it follows that they would have had a lower adiponectin level than patients without MetS.

A linear increase in hs-CRP concentrations with increasing components of MetS has been reported by Laaksonen *et al*.[26] and Lee *et al.*[27] Although hs-CRP concentrations in our patients generally increased with additional criteria, values did not change in a straight line and the differences were not significant. In contrast to a study by Florez *et al*.[28] that showed differences between hs-CRP concentrations and MetS status, we found that hs-CRP concentrations were not significantly different in our patients with and without MetS. In both groups, however, hs-CRP concentrations were elevated to twice the high risk level of 3.0 mg/L, which was indicative of inflammation. A feasible explanation for this finding might be that because patients in both groups had established CAD, they exhibited this underlying feature of the disease. It should be noted that hs-CRP is a non-specific indicator of inflammation[29] and it is possible, therefore, that the relatively higher hs-CRP concentrations were caused by other unknown inflammatory conditions.

Previous studies, including our own contribution to a report on body composition, have stated that BMI and WC are important determinants of increased leptin levels.[30, 31, 32] Linear regression analysis of our total group of patients confirmed that the highest correlation was between leptin and BMI. Of the five MetS criteria, WC correlated strongly with leptin, a result that is consistent with other studies linking obesity with increased leptin levels and an unfavourable metabolic profile.[33, 34] Positive correlations between leptin and hs-CRP, as well as hs-CRP and BMI suggested that these markers were closely related to obesity and inflammation in our CAD patients.

Our study was limited by the relatively small groups of patients and control subjects, and the low number of patients who fulfilled all five criteria for MetS. Although there appeared to be differences of reasonable size in adiponectin and hs-CRP concentrations from one to five criteria, values did not change in a linear manner and this is the likely reason for the lack of statistical significance. It was not our intention to recruit more

men than women, but because CAD is more prevalent in men, the majority of participants in our study happened to be men.

We concluded that leptin, as well as being an indicator of obesity, was also a useful marker of inflammation in our CAD patients. Of the three markers we analyzed, leptin alone was able to differentiate between CAD patients with and without MetS, and to determine MetS status in women and men. Leptin was the only marker that had a significant relationship with increasing criteria for MetS. Thus, in addition to existing MetS criteria, leptin measurements might be helpful in assessing MetS status. Our results confirmed that elevated hs-CRP concentrations indicated an inflammatory state in CAD patients, irrespective of the presence or absence of MetS. Finally, the association of leptin with BMI, WC and hs-CRP revealed a close link between MetS, obesity and inflammation in urban South African black patients with CAD.

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Table 1: Anthropometric measurements and fasting biochemical variables of black coronary artery disease patients with and without the metabolic syndrome, and control subjects

	All CAD patients (n = 40)	*CAD patients with MetS (n = 23)*	*CAD patients without MetS (n = 17)*	*Control subjects (n = 20)*
Age (years)	54.1 ± 8.8	54.4 ± 9.0	53.7 ± 8.6	49.8 ± 9.8
WHR	0.92 ± 0.06	0.93 ± 0.05	0.91 ± 0.08	0.91 ± 0.07
BMI (kg/m^2)	28.2 ± 4.9	30.7 ± 4.8 [b]	24.9 ± 2.7 [d]	29.1 ± 6.8
WC (cm)	96.8 ± 10.5	104 ± 7.2 [b, c]	87.7 ± 6.7	93.5 ± 14.3
Systolic BP (mmHg)	124 ± 15	128 ± 15	119 ± 12	124 ± 14
Diastolic BP (mmHg)	77 ± 9	78 ± 10	76 ± 6	75 ± 8
Triglycerides (mmol/L)	1.7 ± 1.0	1.8 ± 0.9	1.6 ± 1.0	1.3 ± 0.4
HDL-C (mmol/L)	1.2 ± 0.3	1.1 ± 0.3	1.2 ± 0.3	1.3 ± 0.5
Glucose (mmol/L)	5.2 ± 0.8	5.4 ± 0.8 [c]	5.0 ± 0.9	4.6 ± 0.8
Leptin (ng/ml)	11.1 ± 9.6	14.7± 10.8 [a]	6.0 ± 4.0 [d]	18.9 ± 20.0
Adiponectin (*u*g/ml)	19.5 ± 17.2	17.7 ± 17.2	21.4 ± 17.3	19.0 ± 21.5
hs-CRP (mg/L)	6.8 ± 7.4	7.2 ± 7.5 [c]	6.2 ± 7.5	3.6 ± 3.7

Data are expressed as mean ± SD or %.

[a] $p < 0.01$ CAD patients with MetS vs. CAD patients without MetS

[b] $p < 0.0001$ " " " " vs. " " " "

[c] $p < 0.01$ CAD patients with MetS vs. control subjects

[d] $p < 0.02$ CAD " without MetS vs. " "

CAD: coronary artery disease; MetS: metabolic syndrome; WHR: waist-to-hip ratio; BMI: body mass index; WC: waist circumference; HDL-C: high density lipoprotein cholesterol; BP: blood pressure: hs-CRP: high sensitivity C-reactive protein.

Table 2: Marker measurements in black coronary artery disease patients grouped by gender and metabolic syndrome status

	Gender		Women		Men	
	Women (n = 7)	*Men (n = 33)*	*with MetS (n = 6)*	*without MetS (n = 1)*	*with MetS (n = 17)*	*without MetS (n = 16)*
Leptin (ng/ml)	21.5 ± 12.9[a]	8.9 ± 7.2	23.8 ± 12.4[b]	7.6 ± 0.0	11.5 ± 8.4[c]	5.8 ± 4.1
Adiponectin (μg/ml)	13.0 ± 8.9	20.9 ± 18.3	19.1 ± 8.9	21.2 ± 0.0	19.8 ± 11.6	21.4 ± 17.9
hs –CRP (mg/L)	8.5 ± 13.7	6.4 ± 6.0	3.6 ± 2.6	1.0 ± 0.0	6.4 ± 4.0	6.4 ± 7.6

Data are expressed as mean ± SD.

[a] $p < 0.01$ Women vs. men

[b] $p < 0.03$ Women with MetS vs. women without MetS

[c] $p < 0.04$ Men with MetS vs. men without MetS

hs-CRP: high sensitivity C-reactive protein; MetS: metabolic syndrome

Table 3: Marker concentrations at increasing numbers of criteria for the metabolic syndrome in black coronary artery disease patients

Number of MetS criteria	*Leptin (ng/ml)*	*Adiponectin (µg/ml)*	*hs-CRP (mg/L)*
1	3.3 ± 1.6 [a]	26.8 ± 17.1	6.6 ± 5.3
2	8.9 ± 4.7	17.2 ± 11.9	6.7 ± 8.9
3	10.9 ± 8.3	17.9 ± 19.3	7.0 ± 10.5
4	13.4 ± 10.0	23.2 ± 21.8	7.6 ± 5.1
5	21.9 ± 13.7	9.6 ± 2.1	6.3 ± 0.0

Data are expressed as mean ± SD.

[a] $p < 0.05$ Difference in concentrations from 1 to 5 criteria for MetS

hs-CRP: high sensitivity C-reactive protein; MetS: metabolic syndrome

Legend

Fig. 1 Linear regression plots of patients with CAD ($n = 40$) displaying significant positive correlations between **(a)** leptin and body mass index ($r = 0.7107$; $p < 0.0001$); **(b)** leptin and waist circumference ($r = 0.4981$; $p < 0.002$); **(c)** leptin and high sensitivity C-reactive protein ($r = 0.3886$; $p < 0.02$).

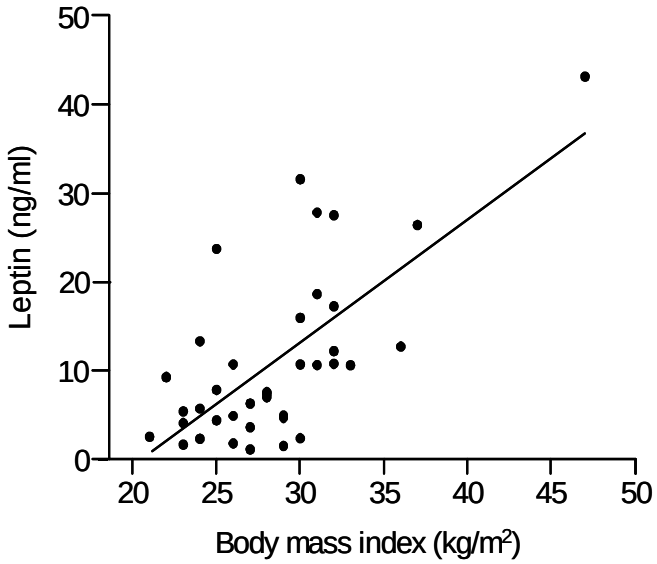
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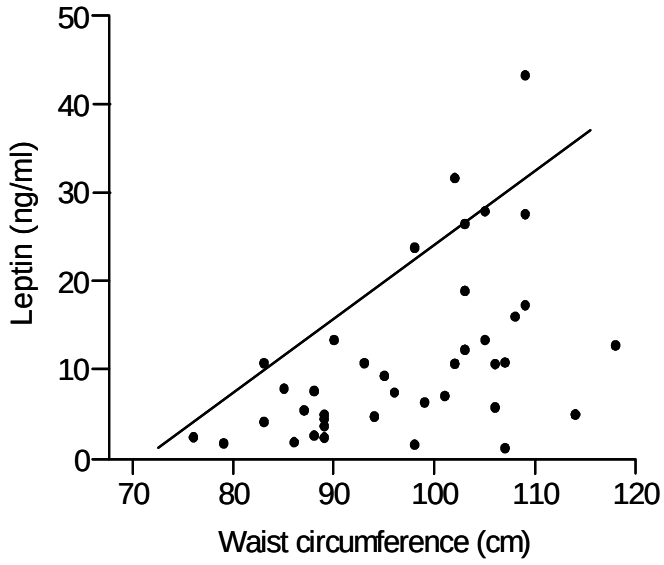
Author Disclosure Statement

Lucas Ntintyane ..... No competing financial interests exist
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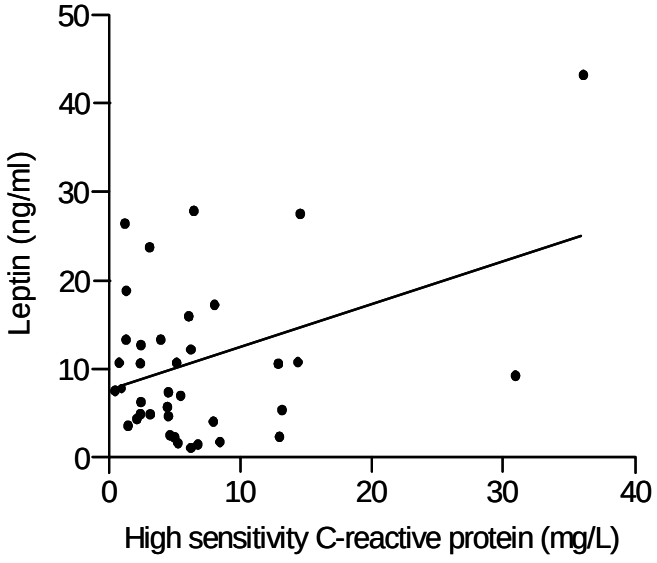
a)


Leptin (ng/ml)
0
10
20
30
40
50
20
25
30
35
40
45
50
Body mass index (kg/m2)

b)


Leptin (ng/ml)
0
10
20
30
40
50
70
80
90
100
110
120
Waist circumference (cm)

c)


Leptin (ng/ml)
0
10
20
30
40
50
0
10
20
30
40
High sensitivity C-reactive protein (mg/L)

APPENDIX I

MANUSCRIPT: DIETARY INTAKE, SOCIO DEMOGRAPHICS AND PHYSICAL ACTIVITY OF A BLACK URBANISED SOUTH AFRICAN POPULATION WITH CORONARY ARTERY DISEASE COMPARED TO HEALTHY VOLUNTEERS

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Dietary intake, socio demographics and physical activity of a black urbanised South African population with coronary artery disease compared to healthy volunteers

ABSTRACT

OBJECTIVE: To investigate the role of diet, socio-demographics and physical activity in a black South African population with coronary artery disease compared to a healthy control group.

DESIGN: Case control study

SETTING: Chris Hani Baragwanath Hospital, Soweto, South Africa

SUBJECTS: Forty black patients with documented CAD and twenty control volunteers who were matched for age, body mass index, waist circumference and waist-hip ratio were included.

OUTCOME MEASUREMENTS: Anthropometrical and biochemical variables as well as socio-demographics, physical activity and quantitative food intake were determined.

RESULTS: No differences were found between the dietary intake, physical activity or socio demographics of the two groups, except for vitamin C intake (p=0.049), with the controls having a median vitamin C intake within the DRI range (94mg) and the CAD patients an intake well below (47mg). Compared to dietary guidelines for prevention of CAD, saturated fatty acid and cholesterol intake were above and fibre, folate and vitamin E all below the recommended intake for both groups. In order to determine the role of urbanisation on the diet, the study population's diet was compared to that of a traditional rural group and an urban group with comparable socio-demographic status. The study population's diet was found to be more westernised compared to both the rural and urban group.

CONCLUSION: When confounding factors, age and obesity were accounted for, there were no differences in the diet, physical activity or socio-demographics of the CAD patients and control group, except for vitamin C intake with both groups having a micronutrient intake below the recommended level. While diet is known to be affected by urbanisation, differences in dietary intakes were observed between the two urban groups, regardless of the similarity in their socio-demographic profile.

INTRODUCTION

The prevalence of cardiovascular disease (CVD) and its risk factors in developed countries has been well documented.[1] However, data is emerging regarding the disconcerting increase in prevalence of CVD in developing countries. A systematic analysis of population health data found that when dividing countries into 2 categories,

that is low and middle income together and then high income countries, Ischemic heart disease (IHD) and cerebrovascular disease (stroke) were the leading cause of death in both of these groups. Together they were responsible for more than one fifth of all deaths worldwide.[2] The large majority of deaths due to IHD (5.7 million of the 7.1 million total deaths) were in the lower and middle-income countries.[2] CVD, particularly coronary artery disease (CAD) was always considered to be rare in the black South African population, but studies are showing an increase in prevalence with urbanisation.[3,4] In the early 1990's approximately 70 black patients with CAD were admitted annually at the Chris Hani Baragwanath Hospital in Soweto (Johannesburg), this has now increased to around 165 cases in 2006.[5] Although there is a definite increase, the increase in the volume of cases has been less than would be expected, compared to the very high prevalence of cardiovascular risk factors in this population.[4,5]

Risk factors such as smoking, elevated low-density lipoproteins (LDL), low high- density lipoproteins (HDL), high blood pressure, elevated glucose, physical inactivity, obesity as well as diet have been proven to be causal risk factors for the development of CVD.[6] Epidemiological, experimental and clinical trial evidence have demonstrated a relationship between diet and many of the other risk factors such as abnormal blood lipid levels, blood pressure, glucose level and obesity.[7,8] For example, diets high in saturated fatty acids, cholesterol and animal fat have been shown to increase LDL cholesterol levels.[8] The westernised diet pattern (higher intake of red meat, processed meat, refined grains, sweets, french fries and high fat dairy products) has been shown to predict incidence of CHD.[9] When looking at the dietary intake in the South African population, there have been shifts in diet intake to a less prudent diet over the past 2 decades.[10] These shifts are occurring at an increased momentum, particularly in the black South African population, where with urbanisation, there has been a definite increase in fat consumption and a decrease in carbohydrate consumption, as has been observed in the North West province of South Africa (THUSA study), as well as in other developing countries.[11,12]. Shifts towards a more westernised diet have however also been observed in rural communities.[10,13] With urbanisation, there is also an increase in socio-economic

status, which is usually accompanied by an increase in other risk factors for CAD, such as obesity and physical activity (PA).[12]

Physical activity has been suggested to be a contributing factor to a decreased risk of CVD, due in part to favourable effects on blood pressure, triglyceride (TG) levels, HDL cholesterol levels, insulin sensitivity and body weight.[14] In South Africa only a few studies have been done to quantify PA patterns in healthy volunteers and to relate PA to CVD risk factors. In the Western Cape, lack of PA was found to be a significant risk factor for non-insulin diabetes mellitus,[15] while in the North West province (THUSA study), physical inactivity was associated with a number of CVD risk factors, especially in overweight black women. Thus indicating the potential role of PA in decreasing CVD risk.[16]

While ample evidence exists regarding the role of dietary intake, PA and socio-demographics in CVD in westernised populations, no data is available on black South Africans with diagnosed CAD. This study therefore investigates the role of diet, socio-demographics and PA in CAD in the black South African population.

METHODS

CAD and control patients

Forty black patients (33 men, 7 women) with documented CAD were included in this study. Coronary artery disease was defined as more than 50% lesions in one or more major coronary arteries, seen with a diagnostic coronary angiogram in the previous 24 months. Subjects with previous myocardial infarction (MI) had to be at least 3 months post-MI before the study. Patients with severe hypercholesterolemia (untreated total cholesterol (TC) of greater than 7.5mmol/L or clinical features of familial hypercholesterolaemia), previously diagnosed diabetes mellitus and who were HIV-positive were excluded. Other exclusion criteria included any overt liver, renal or thyroid disease and smoking in excess of 20 cigarettes per day. Four weeks before the study

started, lipid-lowering medications such as statins and fibrates were discontinued. Any other drugs that might alter lipid levels and/or insulin resistance such as thiazide diuretics, beta-blockers or steroids were stopped three days before tests began. Patients and controls were also asked to refrain from smoking and to fast for 12 hours before the tests.

Twenty volunteers (13 men and 7 women) who were matched with the cases for age, body mass index (BMI), waist circumference (WC) and waist-hip ratio (WHR) were included as a control group. This was done in order to exclude the confounding effects of age and weight differences. The control group had no evidence of coronary atherosclerosis on coronary angiography. The same exclusion criteria, as for the CAD patients, applied for the control group.

STUDY DESIGN

All participants underwent a structured physical examination, and a questionnaire was used to obtain information such as demographic information, medical history, medication use and smoking status. A fasting venipuncture and anthropometrical measurements, which consisted of height, weight and waist and hip circumference was done. Height and weight were measured to the nearest 0.5cm and 0.1kg, respectively, and used to calculate the body mass index (BMI). The waist circumference was measured using a non-elastic measuring tape, at the midpoint between the lowest rib and the iliac crest, to the nearest 0.1cm.

Serum was collected for testing of C-Reactive protein, TC, HDL cholesterol and TG, while plasma for glucose determination was collected in fluoride tubes. Measurements were determined by enzymatic colorimetric methods using a Hitachi automated clinical analyser and reagents were supplied by Roche Diagnostics (GmbH, Mannheim, Germany). Calculation of LDL cholesterol concentrations was based on the Friedewald equation[17]. LDL subfractions were measured in serum by linear, polyacrylamide gel electrophoresis using a Quantimetrix Lipoprint System LDL Subfractions kit

(Quantimetrix, CA, USA). The method resolves the LDL subfractions into profiles consisting primarily of large, buoyant particles or predominantly small, dense particles.

A standardised quantitative food frequency questionnaire (FFQ) was used to determine the dietary intake of the cases and controls.[18] A registered dietician administered the questionnaire; with the aid of "The nutrition transition among the African population of North West" food portion photo book to estimate portion sizes.[19] The nutrient intakes were analysed using the Medical Research Council's FoodFinder3 program, which is based on the South African Food composition tables.[20] A standardised Physical Activity questionnaire[21] was also administered by a registered dietician. Physical activity is reported as Physical Activity Index (PAI). The PAI divides activity into 1 of 3 categories, namely inactive (1- 3.33), moderately active (3.34 – 6.67) and most active (>6.67).[22]

STATISTICAL ANALYSIS

Statistical analysis of data was done using the computer software package Statistica® version 8.[23] Data is reported as median [25 – 75 percentile] for non-parametric data or as mean (Standard deviation) for parametric data. A p-value of less than or equal to 0.05 was regarded as statistically significant. Independent T-tests were done on parametric data and for non-parametric data, the Mann Whitney U test was used when comparing the CAD patients to the control group. For the categorical variables, the Chi-square test was used. Spearmann Rank order correlations were also done to determine associations between risk factors and diet, PA and socio-demographic variables. In order to determine the role of urbanisation on the diet, the dietary intake of this study population were compared to a traditional rural diet and the diet of a comparable urban group with a similar socio-demographic profile. These two communities were both part of the THUSA (Transition, Health and Urbanization in South Africa) study. The THUSA study was a community based, cross-sectional study in the North West Province of South Africa. In this study, dietary intakes of 1751 apparently healthy adult volunteers were obtained using the same validated quantitative FFQ as was used in this study.[24] For the

purpose of this study, the data from the THUSA study was stratified into rural and a comparable urban group (those living in brick houses in townships with the same urbanisation stratum), as well as cases selected that were in the same age group (30 to 70 yrs) and BMI range (20 to 47 kg/m^2) as the study population. Differences between the three groups were determined using ANOVA with Tukey's Honest Significance test for unequal means, as a post-hoc test.

RESULTS

The biochemical characteristics of the study population are shown in Table I. Statistically significant differences were found between the number of smokers, blood pressure, LDL-cholesterol levels, LDL size, as well as fasting glucose and c-reactive protein (CRP) levels of the CAD patients and the control group.

Table 1: Biochemical characteristics of study population

	CAD patients n = 40	**Control n = 20**	**P value**
Gender (men/women)	33/7	13/7	
Age (years)	55 [51 - 61]	49.5 [44.5 – 57.5]	0.07
BMI (kg/m^2)	28 [24.5 - 31]	27.5 [24.5 – 33.5]	0.73
Waist circumference (cm)	98 [88.5 - 106]	94 [83 – 100.5]	0.23
Smoking (n)	14 (35%)	2 (10%)	0.02
Hypertension (n)	38 (95%)	15 (75%)	0.02
Total cholesterol (mmol/L)	5.42 [4.63 – 6.1]	4.63 [3.88 – 5.38]	0.09
HDL cholesterol (mmol/L)	1.14 [0.98 – 1.39]	1.15 [0.95 – 1.41]	0.34
LDL cholesterol (mmol/L)	3.31 [2.64 – 4.07]	2.85 [2.2 – 3.5]	0.005
Triglycerides (mmol/L)	1.38 [1.03 - 2.23]	1.44 [1.02 – 1.64]	0.05
Metabolic Syndrome (n)	24 (60%)	8 (40%)	0.14
LDL size (n with small LDL)	29 (73%)	3 (15%)	<0.0001
Fasting glucose (mmol/L)	5.11 [4.7 – 5.4]	4.6 [3.9 – 5.1]	0.009
Hs-CRP (mg/L)	4.72 [2.48 – 2.32]	2.32 [0.91 – 4.46]	0.026

Data expressed as median [25 – 75 percentile]

CAD: coronary artery disease, BMI: body mass index, HDL: high-density lipoprotein; LDL: low density lipoprotein; CRP: c-reactive protein levels.

The Metabolic Syndrome as classified by the International Diabetes Federation[25] was present in 24 (60%) of the CAD patients compared to 8 (40%) of the controls. Although this is a marked difference, it was not found to be statistically significant. Details pertaining to this high prevalence of Metabolic Syndrome in the CAD cases have been published elsewhere.[5]

Table 2: Comparison of dietary intake between CAD patients, controls and dietary recommendations for prevention of CAD.

	RECOMMENDATIONS	**CAD PATIENTS**	**CONTROLS**
ENERGY (kJ)	Balance calorie intake and physical activity to achieve or maintain a healthy body weight[26]	10470 [9138 – 12462]	10113 [8072 – 11481]
PROTEIN % of TE	± 15 % of TE[26]	12.82 [12.03 – 15.74]	13.22 [12.87 – 15.43]
CARBOHYDRATE % of TE	50 - 60% of TE[27]	55.67 [50.6 – 57.9]	53.45 [48.9 – 56.54]
TOTAL FAT % of TE	25-35% of TE[7]	26.77 [23.74 – 31.67]	28.17 [26.84 – 33.84]
SATURATED FATTY ACIDS % of TE	<7% of TE[26,27,7]	7.45 [6.67 – 9.5]	8.63 [7.23 – 11.36]
TRANS FATTY ACIDS % of TE	< 1%[26,27,7]	0.65 [0.52 – 1.38]	0.96 [0.61 – 1.29]
CHOLESTEROL(mg)	< 200mg[27,7]	298 [265 – 371]	312 [176 – 502]
POLY UNSATURATED FATTY ACIDS % of TE	Up to 10 % of TE[27]	7.2 [5.36 – 8.23]	7.15 [5.82 – 8.36]
MONO-UNSATURATED FATTY ACIDS % of TE	Up to 20 % of TE[27]	9.29 [8.36 – 11.31]	10.21 [8.35 – 10.66]
FIBRE (g)	> 25g per day[27,7]	21.5 [17.3 – 30.7]	20.1 [15.1-26.6]
SUGAR (g)	Minimize intake of foods and beverages with added sugars[26]	64.9[51.2 – 98.5]	55.1 [30.7 – 110.4]

SALT (g)	Chose and prepare food with little or no salt 2.3g/day – sodium[26]	1722 [1362 – 2103]	2070 [1587 – 2593]
ALCOHOL	If you do – in moderation 2 drinks per day – men 1 drink per day – woman[26,7]	0 [0 – 9.4] Men: 1.9 [0.00 – 10.5]* Women:0 [0.00 – 0.00]	0 [0 – 2.4] Men: 1.15 [0.00 – 4.7]* Women:0 [0.00 – 0.00]
SELENIUM (mg)	55 mg[28]	41.6 [30 – 65.2]	40.8 [27 – 57.2]
VITAMIN C (mg)	Men: 90mg Women: 75mg[28]	47 [33 – 81] [a] Men: 45 [33 – 81] Women: 54 [30 – 175]	94 [55 – 140] [a] Men: 79.5 [55 – 142] Women: 94 [58 – 134]
FOLATE (mcg)	400mcg[28]	276 [195 – 344]	221 [207 – 304]
VITAMIN E (mg)	15mg[28]	10.22 [7.19 – 15.97]	11.13 [7.01 – 14.15]
VITAMIN B6 (mg)	Men: 1.7 mg Woman: 1.5mg[28]	1.36 [1.19 – 1.65] Men: 1.37 [1.17 – 1.65] Women: 1.36 [1.19 – 1.77]	1.58 [1.22 - 1.92] Men: 1.49 [1.24 – 1.71] Women: 1.62 [1.09 – 1.92]
B-CAROTENE (mg)	3 – 6 mg[28]	3.31 [2.3 – 4.8]	2.57 [1.36 – 4.73]
PHYSICAL ACTIVITY	> 30 min exercise most days of the week[26]	5.19 [4.21 – 6.29]	4.25 [3.36 – 5.29]
INCOME		3 [3 – 6]	3 [1 – 5.5]
EDUCATION		3 [2 – 5]	3.65 [2 – 5]
HOUSING(brick/ informal)		37/3	19/1

a: P = 0.049 PAI: Physical activity index; % of TE: percentage of total energy

* Equivalent to less than 1 unit of alcohol per day

Income categories: 2:R101-500; 3:R501-1000; 4:R1000-2000.

Education categories: 2:< std 6; 3: Std 6-8; 4: Std 6-8 plus trade; 5: Std 9-10

The macronutrient distribution was within the recommended ranges for prevention of CAD for both groups. Although total fat intake is within recommended ranges, the saturated fatty acid intake and cholesterol intake were above the recommended ranges, while the intake of fibre, folate, selenium, vitamin B6 and E were all below the recommended intake, for both the CAD patients and the healthy volunteers. The vitamin C intake of the healthy volunteers was however within the DRI range (94mg) while that

of the CAD patients was well below (47mg). No correlations were found between biochemical variables and diet, PA or socio-demographic variables.

The dietary intake, PA and socio-demographic information of the CAD patients and control group are reported in Table 2 and compared to the dietary guidelines for the prevention of CAD. The guidelines were compiled using various existing dietary guidelines, to ensure that all variables would have a guideline for comparison.[7,26,27,28] When comparing the dietary intake of the CAD patients to the control group, the only nutrient that was found to be significantly different was the vitamin C intake ($p=0.049$). No differences were found in PA, income, and type of housing or education level between the groups. Both the CAD patients and the control group had a median PAI that fell in the moderately active category for PA.

The dietary intake of the study population is compared to the THUSA urban and rural groups in Table 3. The mean dietary intake of our study population was significantly higher than that of both the rural and urban groups for energy, percentage of protein and carbohydrate of total energy, as well as dietary fibre and vitamin C. The percentage of energy coming from total fat, saturated fat and mono-unsaturated fat did not differ between the THUSA urban and the study population, but were however significantly higher compared to the THUSA rural group.

When looking at PA, both the THUSA rural and urban groups had a mean PAI level that fell in the inactive category, while the study population's mean PAI fell in the moderately active group. Significant differences were found for income, between all 3 groups, with the study population being in the highest category.

Table 3: Comparison of dietary intake, physical activity and socio-demographic information of study population to rural and urban strata from THUSA study.

	THUSA Rural (n = 250)	THUSA Urban (n = 248)	Study population (n = 60)	P value
Energy (kJ)	8368.7±3519.5[a]	8511±3469.17[b]	10752±2952.71[ab]	<0.001
Protein (% of TE)	12.03±2.22[ab]	12.66±2.47[ac]	13.92±2.45[bc]	<0.001
Fat (% of TE)	22.86±7.09[ab]	27.36±7.50[a]	27.64±5.60[b]	<0.001
Carbohydrate (% of TE)	65.13±9.36[ab]	58.76±10.03[ac]	53.91±5.19[bc]	<0.001
Saturated fat (% of TE)	6.98±3.08[ab]	8.42±2.97[a]	8.41±2.29[b]	<0.001
MUFA (% of TE)	7.45±2.68[ab]	9.48±2.99[a]	9.69±2.39[b]	<0.001
PUFA (% of TE)	6.25±2.18[a]	6.98±2.22[a]	7.07±2.00	0.039
Total trans fatty acids (g)	1.17±0.94[ab]	1.63±1.41[a]	1.19±1.05[b]	<0.001
Cholesterol (mg)	269.82±194.39[a]	340.92±210.74[a]	349.79±191.42	0.011
Alcohol (g)	7.98±26.71	12.02±(39.61)	4.42±8.66	0.4
Total dietary fibre (g)	16.47±8.15[a]	17.33±8.34[b]	23.14±9.12[ab]	<0.00001
Vitamin C (mg)	30.31±31.11[ab]	40.37±41.17[ac]	86.27±69.49[bc]	<0.001
Vitamin E (mg)	10.10±4.94	11.57±6.81	12.08±6.07	0.29
Income	2.41±1.1[ab]	2.933±1.32[ac]	3.58±1.9[bc]	<0.0001
Physical activity (PAI)	2.43±0.76[a]	2.72±1.10[b]	5.33±2.29[ab]	<0.001

TE: Total energy; MUFA: mono-unsaturated fatty acids; PUFA: poly-unsaturated fatty acids; PAI: physical activity index

Data presented as mean±SD

Mean values with the same superscript letter differ significantly $p \leq 0.05$ determined with post-hoc tests

Income: Categories: 2:R101-500; 3:R501-1000; 4:R1000-2000.

DISCUSSION

The definite increase in the known risk factors for CAD, as well as the increase in CAD observed in the black South African urbanised population prompted this study. In order to determine the role of the diet in this increased prevalence of both the risk factors and the disease, the dietary intake of age and BMI matched CAD patients and a control group

were compared. No significant difference in diet, PA and socio-demographics was found between the CAD patients and control group, except for vitamin C intake.

When comparing the two groups to the dietary guidelines for prevention of CAD, the results suggest that the study population as a whole possibly has a suboptimal fruit and vegetable intake (due to low fibre and micronutrient intake), as well as a saturated fat and cholesterol intake above the recommended intake. This trend was also seen in the INTERHEART study, results of which suggest that increased consumption of fruit and vegetables and a reduced intake of fried foods, probably related to type of fat used for frying and salty snacks is likely to reduce the risk of MI in all regions of the world.[29]

The INTERHEART Africa study found that known CVD risk factors account for ≈ 90% of MI observed in African populations (smoking history, diabetes history, hypertension, abdominal obesity and ratio of apolipoprotein B to apolipoprotein A-1).[12] Evidence from prospective studies have also shown that dietary patterns are associated with increased risk for CHD.[8] It has also been observed that there have been shifts in dietary intake of the black South African population over the past 2 decades to a more westernised diet.[10,13] Appropriate fibre, folate, vitamin E and vitamin C intake have been associated with a reduced cardiovascular risk in prospective studies, however when the effect of some of these nutrients, for example their anti-oxidant capacity were tested individually in clinical trials, the results were not significant.[30] This has led to a shift from looking at individual nutrients and supplementation to rather looking at food-based hypotheses and the cardioprotective properties of fruit and vegetables as a whole.[31,32] The consumption of fruit and vegetables, particularly green leafy vegetables and vitamin C rich fruit and vegetables appears to have a protective effect against CHD.[32,33,34]

The macronutrient intake of both the CAD patients and the controls compared well with the guidelines for the prevention of CAD, with only saturated fatty acids and cholesterol being higher than the guideline, although only marginally for both groups. These results suggest that for this population, the development of CAD was not related to increased macronutrient intake. The micronutrient intake for both groups was below the

recommendations, except the vitamin C intake of the healthy volunteers, which was within the DRI range (94mg). Vitamin C, besides being an antioxidant also promotes fast regeneration of the endothelium after tissue injury, which is of importance during secondary prevention as well as early development of CAD.[35] This suggests that while no differences exist in the macronutrient intake between the two groups, difference in micronutrient intake may contribute to the development of CAD. This is supported by the fact that in the INTERHEART study, a low fruit and vegetable intake (as rich sources of micronutrients) was found to be one of the leading causes of CAD. [12] There is however, also the possibility that the control group may still develop CAD. Although not significantly so, the control group (49.5 years) had a younger median age than the CAD patients (55 years). These ages are similar to the ages that were reported in the INTERHEART Africa study for control subjects (50.6 years) and those that have developed CAD (53 years).[12] It is a possibility that by the time the controls reach the same age as the cases, they may also develop CAD.

When this study population's dietary intake, PA and socio-demographics were compared to another South African population group, some differences were found. As would be expected based on their level of urbanisation, their dietary intake, income and PA differed from the traditional rural group. The higher fat consumption in our study population is consistent with other findings[10] that show that there is an increase in fat intake with urbanisation, however the percentage fat intake is still well within the recommended intake and not as high as the typical westernised diet.[10] The study populations' dietary intake was however also higher for most nutrients than the THUSA urban group. Despite having the same socio-economic status and being in the same level of urbanisation, the study population fell in a higher income category than the THUSA urban group. Maize meal was the staple food with a low fruit and vegetable intake[24] common in all three groups. However, in both the THUSA rural and urban group, more than half the protein intake was derived from plant sources, while the opposite was found in this study population with almost 60% coming from animal sources.

When looking at PA, both the rural and urban groups from the THUSA study fell in the inactive category, while the study population fell in the moderately active group. The low PA in the THUSA group may be explained by the fact that traditionally living rural people do not expend energy without a purpose, namely to perform a task.[16] In our study population, few owned their own form of transport and spent energy walking to taxi ranks or to work, which possibly explains their higher PA level and possibly contributes to the observed increased dietary intake.

A limitation of this study was the fact that the CAD patients had to be at least 3 months post MI before being included in this study. This could have resulted in the patients having changed their habitual dietary intake in the interim, as dietary education is a standard part of the hospital treatment following a MI.

CONCLUSION

Little is known about the causes of CAD in the black South African population. When confounding factors age and obesity were accounted for, there were no differences in the diet, physical activity or socio-demographics of the CAD patients and control group, except for vitamin C intake. The macronutrient intake of both groups was within recommendations, while both groups had a micronutrient intake below the recommended level. While diet is known to be affected by urbanisation, differences in dietary intakes were observed between the two urban groups, regardless of the similarity in their socio-demographic profile.

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