CHAPTER 3

RISK FACTOR PROFILE OF CORONARY ARTERY DISEASE IN BLACK SOUTH AFRICANS

M. Pieters[1], R. Dolman[1], L Ntyintyane[2], J. Jerling[1] & F. Raal[2]

[1]Centre of Excellence for Nutrition, North West University, South Africa

[2]Carbohydrate and Lipid Metabolism Research Unit, University of Witwatersrand, South Africa

Published in *South African Heart Journal* 2011, 8:4-11 (Annexure A).

INSTRUCTIONS FOR AUTHORS FOR THE SOUTH AFRICAN HEART JOURNAL

SA HEART IS AN OFFICIAL JOURNAL OF THE SOUTH AFRICAN HEART ASSOCIATION AND APPEARS ON A QUARTERLY BASIS

Instructions for authors

SA Heart publishes peer reviewed articles dealing with cardiovascular disease, including original research, topical reviews, state-of-the-art papers and viewpoints. Regular features include an ECG quiz, image in cardiology and local guidelines. Case reports are considered for publication only if the case or cases are truly unique, incorporates a relevant review of the literature and makes a contribution to improved future patient management.

Publication policy

Articles must be the original, unpublished work of the stated authors. Written permission from the author or copyright holder must be submitted with previously published material including text, figures or tables. Articles under consideration elsewhere or previously published (except as abstracts not exceeding 400 words) may not be submitted for publication in SA Heart. On acceptance transfer of copyright to the South African Heart Association will be required. No material published in SA Heart may be reproduced without written permission. Permission may be sought from the Editor (email: afd@sun.ac.za).

Disclosures

Authors must declare all financial disclosures and conflicts of interest in the cover letter and on the title page of the manuscript.

Ethics

All studies must be in compliance with institutional and international regulations for human and animal studies such as the Helsinki declaration (2008) (http://www.wma.net/en/30publications/10policies/b3/17c.pdf) and the South African MRC ethics guidelines (http://www.sahealthinfo.org/ethics/index.htm). Human studies require ethics committee approval and informed consent which must be documented in your manuscript. Animal studies require ethics committee approval and must conform to international guidelines for animal research. Compliance with these requirements must be documented in your manuscript.

Content

1. Title page: It should contain the title of the manuscript, the names of all authors in the correct sequence, their academic status and affiliations. If there are more than 4 authors, the contribution of each must be substantiated in the cover sheet. The main author should include his/her name, address, phone, fax and email address.

2. Authors are solely responsible for the factual accuracy of their work.

3. Articles should be between 3 000 and 5 000 words in length.

4. A 200-word abstract should state the main conclusions and clinical relevance of the article.

5. All articles are to be in English.

6. Abbreviations and acronyms should be defined on first use and kept to a minimum.

7. Tables should carry Roman numeral, I, II etc., and figures Arabic numbers 1, 2 etc.

8. References should be numbered consecutively in the order that they are first mentioned in the text and listed at the end in numerical order of appearance. Identify references in the text by Arabic numerals in superscript after punctuation, e.g. …trial.[13]

The following format should be used for references:

Articles

Kaplan FS, August CS, Dalinka MK. Bone densitometry observation of osteoporosis in response to bone marrow transplantation. Clin Orthop 1993;294:73-8. If there are more than six authors, list only the first three followed by et al.

Chapter in a book

Young W. Neurophysiology of spinal cord injury. In: Errico TJ, Bauer RD, Waugh T (eds). Spinal Trauma. Philadelphia: JB Lippincott; 1991: 377-94.

Online media

Norback JS, Lwellyn DC and Hardin JR (2001). Shoptalk 101. Integrating workplace communication into undergraduate engineering curricula [online]. Retrieved February 15, 2012: http://www.lionhrtpub.com/orms/orms-8-01/norback.html.

9. Articles are to be submitted by email. The text should be in MS Word. Pages should be numbered consecutively in the following order wherever possible: Title page, abstract, introduction, materials and methods, results, discussion, acknowledgements, tables and illustrations, references.

10. Where possible all figures, tables and photographs must also be submitted electronically. The illustrations, tables and graphs should not be imbedded in the text file, but should be provided as separate individual graphic files, and clearly identified. The figures should be saved as a 300 dpi jpeg file. Tables should be saved in a MS Word or PowerPoint document. If photographs are submitted, two sets of unmounted high quality black and white glossy prints should accompany the paper. Figures and photographs should be of high quality with all symbols, letters or numbers clear enough and large enough to remain legible after reduction to fit in a text column. Each figure and table must have a separate self-explanatory legend.

11. Remove all markings such as patient identification from images and radiographs before photographing.

Submission of manuscripts

Please submit the manuscript to the Editor (afd@sun.ac.za) with copies to the Guest Editor (if applicable) and the secretary of the South African Heart Association (erika@saheart.org).

ABSTRACT

Objectives: The aim of this study was to investigate the risk factor profile of coronary artery disease (CAD) in black South Africans. The study was motivated by the increased prevalence of CAD in South Africa, probably as a result of urbanisation. Despite this increase, however, very little is known regarding the cause, risk factor profile and clinical presentations of CAD in the black South African population.

Design: A case control study was performed investigating 40 (33 men, 7 women) angiographically defined CAD patients and 20 (13 men and 7 women) age and body composition matched controls.

Results: There was no difference in physical activity, socio-demographic factors or dietary intakes between the CAD and control group, except for the CAD patients consuming less vit C (40.9 vs 61.3 mg). The CAD group had significantly higher LDL-C, fasting glucose and CRP. There was also a significantly higher prevalence of smokers (35 vs 10%), hypertension (95 vs 75%) small dense LDL (73 vs 15%) and insulin resistance (M-value of 4.15 vs 12.5 mg/kg/min) in the CAD compared to the control group. In a logistic regression model, small dense LDL and insulin resistance were the main predictors of CAD.

Conclusions: Black South African CAD patients had increased levels of the same risk factors that are typically seen in Caucasians with insulin resistance and small dense LDL being particularly significant in their contribution.

Keywords: Coronary artery disease, Africans, CVD risk factors, diet, LDL size, insulin resistance

INTRODUCTION

While age-adjusted cardiovascular disease (CVD) death rates have declined in several developed countries, rates of CVD have increased disconcertingly in low- and middle-income countries.[1,2] Coronary artery disease (CAD) specifically, has historically been remarkably rare in black South Africans, but studies are now showing an increase in prevalence especially in urban areas as a result of urbanisation.[3-5]

Recognized risk factors that have been shown to be affected by urbanisation in the Transition and Health during Urbanization in South Africans (THUSA) study include increases in hypertension, obesity, smoking habit and hyperfibrinogenemia.[6] Changes in dietary intakes during urbanization are considered to play a prominent role in the observed increase in risk factors.[7] With urbanisation, there is also an increase in socio-economic status, which is usually accompanied by an increase in other risk factors such as obesity and physical inactivity.[8]

Very little is, however, known regarding the cause, risk factor profile and clinical presentations of CAD in the black South African population. Steyn et al.[8] determined in the INTERTHEART study that 89.2% of the risk for an initial myocardial infarct in Africans can be accounted for by five risk factors namely: current/former tobacco smoking, self-reported hypertension and diabetes, abdominal obesity measured as waist to hip ratio and lipoprotein ApoB/ApoA-1 ratio. Contrasting gradients were however, found in socioeconomic class, risk factor patterns and myocardial infarction risk between different ethnic groups.

It is not yet known what the individual contribution of each of the known risk factors for CAD development in black Africans is. Differences for instance occur in the prevalence of individual risk factors in black Africans compared to Caucasians such as hypertension and the lipid profile. The African population is known to be especially vulnerable to hypertension.[9] Kearney et al.[10] predicted that by 2025, 73.6 million men and 77.1 million women in Sub-Saharan Africa will be hypertensive with urbanisation significantly contributing to this increased prevalence.[7] Black South Africans in general, on the other hand seem to have a favourable lipid profile with lower total cholesterol (TC) and higher high density lipoprotein cholesterol (HDL-C) levels than other ethnic groups in South Africa.[6] Black Africans with heart disease in the Heart of Soweto study also had lower cholesterol levels than other ethnicities.[5] Nethononda et al.[11] furthermore demonstrated that black South African CAD patients in their study, had cholesterol levels within the target range recommended by the adult treatment panel III (ATPIII) guidelines of the National Cholesterol Education Programme (NCEP), placing a question on the role of cholesterol lowering in CAD in black Africans.

Therefore, due to the increase in prevalence of CAD and to better understand the risk factor profile and pathophysiology thereof in black South Africans, we undertook a study to compare dietary intakes, physical activity level, socio-demographic background and biochemistry of angiographically defined black South African CAD patients with a control group from a similar socio-demographic background. We excluded the complex and confounding effects of diabetes and obesity by excluding diabetic patients and by matching CAD cases and controls for not only body mass index (BMI) but also for waist circumference and waist-hip ratio.

METHODS

Study population

Forty black patients (33 males, 7 females) with documented CAD who attended the Chris Hani Baragwanath hospital in Soweto were included after signing informed consent (Annexure B). Ethical approval was obtained from the Wits Health Consortium (No: 010102). 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 TC of > 7.5mmol/L or familial hypercholesterolaemia), previously diagnosed diabetes mellitus or who were HIV-infected 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 sample collection. Patients and controls were also asked to refrain from smoking for 12 hours prior to sample collection.

Twenty black healthy volunteers (13 men and 7 women) from a similar socio-demographic background and who visited the cardiac clinic of the same hospital, matched for age, BMI, waist circumference and waist-hip ratio 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.

MATERIALS AND METHODS

Demographic information, medical history, medication use and smoking status were obtained using questionnaires (Annexure C). A standardised and validated quantitative food frequency questionnaire (Annexure D), developed for the African population, together with a food portion photo book were used to determine dietary intakes.[12,13] The nutrient intakes were analysed using the Medical Research Council's FoodFinder3 program, which is based on the South African Food composition tables.[14] A standardised Physical Activity questionnaire[15] (Annexure E) was used to calculate the Physical Activity Index. Anthropometrical measurements consisted of height, weight and waist and hip circumference. Height and weight were used to calculate BMI.

Fasting blood samples were collected by a qualified nursing sister. Serum was prepared for C-Reactive protein (hs-CRP), TC, HDL-C, triglycerides (TG), Apo A1, Apo B, Lp(a), pro-insulin, insulin, C-peptide, adiponectin, leptin, free fatty acids (FFA),and uric acid while plasma for glucose determination was collected in fluoride tubes. Urine was collected for measurement of urinary albumin. Blood samples were centrifuged at 1500g, for 20 min within 1 hour of collection and then stored at -70°C until analysis.

Glucose, TG, TC, HDL-C, hs-CRP, uric acid and urinary albumin were determined by enzymatic colorimetric methods using a Hitachi automated clinical analyser and reagents (Roche Diagnostics GmbH, Mannheim, Germany) in a routine laboratory. Low density lipoprotein cholesterol (LDL-C) concentrations were calculated using the Friedewald equation.[16] LDL subfractions were measured in serum by linear, polyacrylamide gel electrophoresis using a Quantimetrix Lipoprint System LDL Subfractions kit (Quantimetrix, CA, USA). Apo A1, Apo B and Lp(a) were analysed using immunoturbidimetric assays (Tina-quant, Roche Diagnostics GmbH, Mannheim, Germany). Insulin and c-peptide were analysed using chemiluminescent immunometric assays (IMMULITE, Siemens Medical Solutions Diagnostics Ltd, Gwynedd, UK). Proinsulin was analysed using an enzyme linked immunosorbend assay (ELISA) (Dako-Cytomation Ltd, Cambridgeshire, UK). Leptin and adiponectin were analysed with sandwich ELISA's (Quantikine Immunoassays, R & D Systems, Minneapolis, USA). Free fatty acids were determined with an enzymatic colorimetric assay (NEFA, Roche Diagnostics GmbH, Mannheim, Germany). Insulin-mediated glucose disposal (M-value) was determined using the hyperinsulinaemic euglycaemic clamp technique and expressed as mg/kg/min with a normal value being > 5.0mg/kg/min and a value below this indicating insulin resistance.[17] Intima media thickness (IMT) was measured using B-mode ultrasound at the optimum angle of interrogation at the

flow tip divider, the common carotid artery, external carotid artery and internal carotid artery at the bifurcation as described in detail by Holland et al.[18]

Statistical analysis

Statistical analysis of data was done using the computer software package Statistica® version 8. Data is reported as median [25 – 75 percentile] for non-parametric data or as mean (standard deviation) for parametric data. A p-value ≤ 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. Analysis of Co-Variance (ANCOVA) was used to determine differences between the CAD and control group after adjusting for possible confounding effects of age. Only variables that were found to be affected by age were adjusted for age. For the categorical variables, the Chi-square test was used. Spearman Rank order correlations were done to determine associations between risk factors and diet, physical activity and socio-demographic variables. Logistic regression was used to determine predictors for categorical variables such as LDL-size and CAD.

RESULTS

The clinical and biochemical characteristics of the study population are shown in Table I. The CAD patients had significantly higher median LDL-C, fasting glucose, CRP and significantly lower TG and M-values than the control group. The median M-value of the CAD group was 4.15 mg/kg/min, indicating the presence of insulin resistance.[17] Although not significantly so, TC was higher in the CAD than in the control group (5.42 vs 4.63 mmol/L). The TC level of the CAD group was furthermore higher than the target range (<5.2mmol/L) recommended by the ATP III criteria of NCEP.[19] LDL-C was higher than the ATPIII target range in both groups (< 2.59mmol/L). The CAD group had non-significantly higher IMT than the control group (1.1 vs 0.93mm). Forty five percent of the control subjects and 70% of the CAD patients had increased IMT, using 0.8mm as cut-off.[20] IMT correlated significantly with age (r=0.47; p=0.0005), CRP (r=0.45; p=0.002) and fasting plasma glucose (r=0.29; p=0.46). Adjusting for age affected only CRP, leptin and IMT. After adjustment for age, CRP was no longer significantly different between the CAD and control group (p=0.2), while leptin levels were now significantly lower in the CAD than the control group (p=0.023), the difference in IMT remained non-significant (p=0.97).

Table I: Clinical and biochemical characteristics of study population

	CAD patients n = 40	Control n = 20	P value
Gender (males/females)	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]	0.041
Triglycerides (mmol/L)	1.38 [1.03 - 2.23]	1.44 [1.02 – 1.64]	0.05
LDL size (n with small LDL)	29 (73%)	3(15%)	<0.0001
Apo A1 (mg/dL)	133.1 [104.8 – 148.3]	130 [106.2 - 141.5]	0.30
Apo B (mg/dL)	101.3 [76.5 – 115.1]	83.9 [72.7 – 99.7]	0.11
Apo B / Apo A1	0.82 [0.58 – 0.91]	0.66 [0.57 – 0.82]	0.18
Lp(a) (mg/dL)	51.45 [39.6 – 76.7]	56.1 [24.4 – 71.1]	0.48
Metabolic Syndrome (n)	24(60%)	8 (40%)	0.14
Fasting glucose (mmol/L)	5.11 [4.7 – 5.4]	4.6 [3.9 – 5.1]	0.009
Insulin (mIU/L)	5.29 [3.29 – 12.2]	3.93 [2-6.32]	0.10
M-value (mg/kg/min)	4.15 [3 – 5.15]	12.5 [5.75 – 14.15]	<0.0001
Proinsulin (pmol/L)	1.31 [0.76 – 2.86]	1.73 [0.75 – 2.58]	0.72
C-peptide (μg/L)	1.1 [0.69 – 1.51]	0.62 [0.4 – 1.41]	0.22
Hs-CRP (mg/L) [a]	4.72 [2.48 – 8.00]	2.32 [0.91 – 4.46]	0.026 (0.2)
Adiponectin (mg/mL)	11.32 [5.91 – 30.45]	12.38 [7.85 – 18.53]	0.88
Leptin (ng/mL) [*]	7.71 [4.25 – 13.33]	12.9 [3.82 – 19.78]	0.35 (0.023)
Free fatty acids (mmol/L)	0.84 [0.63 – 1.04]	0.85 [0.76 – 1.06]	0.76
Uric acid (mmol/L)	0.39 [0.35 – 0.45]	0.43 [0.34 – 0.48]	0.42
Urinary albumin (mg/L)	6 [2.9 – 20.4]	4 [0.4 – 16]	0.458
Intima media thickness (mm) [*]	1.1 [0.82 – 1.32]	0.93 [0.6 – 1.43]	0.16 (0.97)

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. p value in brackets after adjustment for age

There was a significantly higher prevalence of smokers (35 vs 10%), hypertension (95 vs 75%) and small dense LDL (73 vs 15%) in the CAD compared to the control group. When subdividing the population based on LDL-size, 90% of the subjects with small dense LDL were in the CAD group. The subjects who did not smoke had a similar distribution of small dense and large buoyant LDL (46 vs 54%) while in the smokers, 87% of subjects had small dense LDL. A similar trend can be seen for metabolic syndrome. Patients without the metabolic syndrome had a similar distribution of small dense and large LDL (48 vs 52%) while 66% of those with the metabolic syndrome had small dense LDL, compared to 33% who had large buoyant LDL. Using logistic regression, age and M-value were the only predictors of LDL-size and the subjects with large buoyant LDL had a significantly higher M-value than the subjects with small dense LDL (8 vs 3.8 mg/kg/min) who, based on the M-value were considered to be insulin resistant.

The metabolic syndrome as classified by the International Diabetes Federation,[21] 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 different. Details pertaining to this high prevalence of metabolic syndrome in the CAD cases have been published elsewhere.[22]

Using a logistic regression model that included the risk markers that differed between the CAD and control group, M-value, CRP and LDL size were the main predictors of CAD.

The dietary intake, physical activity and socio-demographic information of the CAD patients and control group are reported in Table II and compared to the dietary guidelines for the prevention of CAD. The only nutrient that was found to be significantly different was the vitamin C intake ($p=0.049$) with CAD patients having a significantly lower intake than the control group. No differences were found in physical activity, income, and type of housing or education level between the groups. Both the CAD patients and the control group had a median Physical Activity Index that fell in the moderately active category.

Both the CAD and the control group had a relatively high total energy intake (±10 000kJ) in comparison with their physical activity as can be seen by their increased median BMI (28 and 27.5 kg/m^2). In general, the macronutrient distribution was within and the micronutrient intakes below the recommended ranges for prevention of CAD for both groups. Although total fat intake was within recommended ranges, the saturated fatty acid intake was above the recommended ranges and cholesterol intake was at the upper limit, while the intake of fibre, folate, selenium vitamin B6, vitamin C and E were all below the recommended intake, for both the CAD patients and the control group. The women in both the control and CAD group did not consume any alcohol.

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

Nutrient	Recommendations	Cad patients	Controls
Energy (kJ)	Balance calorie intake and physical activity to achieve or maintain a healthy body weight[42]	10566 [9156 – 13299]	9630 [7725 – 12716]
Protein % of TE	≈ 15 % of TE[42]	12.98 [12.04 – 15.7]	13.46 [12.49 – 15.09]
Carbohydrate % of TE	50 - 60% of TE[19]	53.52±5.93	53.08±4.89
Total fat % of TE	25-35% of TE[43]	27.75±5.99	29.29±4.91
Saturated fatty acids % of TE	<7% of TE[19;42;43]	8.34±2.05	9.05±2.42
Trans fatty acids % of TE	< 1%[19;42;43]	0.24 [0.14 – 1.58]	0.47 [0.27 – 0.83]
Cholesterol (mg)	< 300mg[42]	315.7 [267.4 – 420.7]	289.7 [187.4 – 523.1]
Poly unsaturated fatty acids % of TE	Up to 10 % of TE[19]	6.96±2.17	7.84±2.37
Mono-unsaturated fatty acids % of TE	Up to 20 % of TE[19]	9.85±2.70	9.38±1.79
Fibre (g)	> 25g per day[19;43]	21.73 [17.06 – 30.6]	17.31 [14.43-26.23]
Added sugar (g)	Minimize intake of foods and beverages with added sugars[42]	66.13[49.04 – 97.41]	63.77 [33.57 – 86.83]
Sodium (g)	Chose and prepare food with little or no salt 2.3g/day – sodium[42]	1.81±0.62	2.04 ± 0.58
Alcohol (g)	If you do – in moderation 2 drinks per day – men 1 drink per day – woman[42;43]	0 [0 – 10.49] Men: 2.5 [0.00 – 10.49] * Women:0 [0.00 – 0.00]	0 [0 – 2.63] Men: 1.21 [0.00 – 4.66] * Women:0 [0.00 – 0.00]
Selenium (mg)	55 mg[19]	40.21 [31.43 – 60.3]	45.57 [29.4 – 59.31]
Vitamin C (mg)	Male: 90mg Female 75mg[44]	40.9 [30.3 – 66.9]† Men: 41 [29.9 – 66.9] Women: 39 [34.7 – 183]	61.3 [50.4 – 125] † Men: 64.4 [54.9 – 143] Women: 61.3 [41.4 – 97]
Folate (μg)	400μg[44]	272.4±105.42	244±69.44
Vitamin E (mg)	15mg[44]	10.68 [8.07 – 15.93]	12.71 [7.92 – 15.6]
Vitamin B6 (mg)	Male: 1.7 mg Female: 1.5mg[44]	1.38 [1.12 – 1.78] Men: 1.4 [1.12 – 1.78] Women: 1.34 [1.06 – 1.87]	1.55 [1.17 - 1.88] Men: 1.52 [1.29 – 1.68] Women : 1.57 [1.08 – 1.88]
B-carotene (mg)	3 – 6 mg[44]	3.09 [1.79 – 4.39]	2.55 [1.42 – 4.28]
Physical activity index ‡	> 30 min exercise most days of the week[42]	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

Data expressed as median [25 – 75 percentile] or mean±standard deviation; * Equivalent to less than 1 unit of alcohol per day; †: P = 0.049; % of TE: percentage of total energy; ‡ Physical activity index: 1- 3.33: inactive; 3.34 – 6.67: moderately active; >6.67 most active; § Income categories: 2:R101-500; 3:R501-1000; 4:R1000-2000; 5:R2000-R3000; 6:>R3000; ¯ Education categories: 2:< std 6; 3: Std 6-8; 4: Std 6-8 plus trade; 5: Std 9-10.

DISCUSSION

The purpose of this study was to help determine the risk factor profile and clinical presentations of CAD in black South Africans by excluding the confounding metabolic derangements that accompany the known risk factors, diabetes and obesity. The CAD patients had a higher prevalence of smoking, hypertension, small dense LDL and metabolic syndrome with specific emphasis on insulin resistance as well as increased LDL-C, TC, CRP and fasting glucose than the controls. These factors are all documented to be involved in atherosclerosis.[23-25] The decreased vitamin C intake together with increased smoking may confer additional risk through increased oxidative stress.[26]

The main predictors of the development of CAD in this black South African population were small dense LDL and insulin resistance. While more CAD patients had metabolic syndrome (60%), compared to the control group (40%), it was insulin resistance *per se* that seemed to be the major distinguishing factor. By excluding overt diabetes and matching for body fat distribution, it was possible to determine the independent contribution of insulin resistance amongst the many components of the metabolic syndrome. Insulin resistance as well as the resultant hyperglycaemia contribute to atherosclerosis through several mechanisms including modification of the lipid metabolism to produce a pro-atherogenic lipid profile, inflammatory signalling pathways such as NF-kB, MAP kinase and protein kinase C, direct effects on the vasculature, oxidative/mitochondrial stress and genomic stress.[27,28] The lower leptin levels observed in the CAD group may furthermore facilitate the insulin resistance seen in this group. Leptin is considered to improve peripheral insulin sensitivity and modulates pancreatic β–cell function.[29,30]

Small dense LDL confer atherogenic risk through increased trans-endothelial transport, increased susceptibility to oxidation, reduced LDL receptor affinity, increased binding to intimal proteoglycans and increased formation of proaggregatory and vasoconstrictor mediators.[31-33] Some controversy still exists, however regarding the independent predictive role of small dense LDL in CVD. The vast majority of both cross-sectional and prospective epidemiological studies have indicated a significant association between small, dense LDL and increased coronary heart disease risk. Only some studies, however, found it to be an independent predictor once adjusting for confounding variables such as increased plasma TG and decreased HDL-C levels, [34,35] that frequently accompany small, dense LDL. The

CAD patients in this study did, however, not have decreased HDL-C nor increased TG, while 75% had small, dense LDL, suggesting that in the black South African population, LDL-size may independently confer additional risk.

From the results it seems that insulin resistance and to a lesser extent, smoking are strongly related to the presence of small dense LDL. Insulin resistance has also in the literature been shown to be strongly related to LDL size.[36,37] It is suggested that in insulin resistance, non-esterified fatty acids released by adipocytes provide more triglyceride for VLDL production as a result of a lack of inhibition of hormone-sensitive lipase resulting in the production of small dense LDL.[38] Insulin resistance furthermore expedites cholesteryl ester transfer protein-mediate exchange of LDL cholesterol ester for VLDL triglycerides. This newly acquired LDL triglyceride undergoes lipolysis by hepatic and lipoprotein lipase, to form small dense LDL.[38,39] It is therefore possible that the high prevalence of small dense LDL present in the CAD patients may, at least in part, be caused by the insulin resistance. From the results it is also clear that while non-smokers had an equal distribution of LDL particle size, smokers had a significantly higher prevalence of small dense LDL than large buoyant LDL. This suggests that smoking may contribute to the development of small dense LDL. Very little data is available regarding this in the literature and well controlled intervention studies are required to determine whether smoking can alter LDL size.

In contrast with the results of Nethononda et al.,[11] the CAD patients in this study had both moderately increased TC and LDL-C levels. The controls did however also have increased LDL-C. This may be the result of the health transition associated with urbanisation affecting the previously reported protective lipid profile of black South Africans.

The CAD patients had a significantly lower intake of vit C than the controls, most probably reflecting a lower intake of fresh fruit and vegetables. No other differences were observed in the dietary intakes between the CAD patients and controls having similar macro- and micronutrient distributions. It is however possible that while current diets of the patients and controls did not differ significantly, differences in dietary intakes during earlier stages of life may have been involved in the early development of atherosclerosis. It should also be considered that differences in dietary intakes are not only detected by use of nutrient analysis but also by use of food consumption patterns. Nutrient analysis should therefore be used in conjunction with food consumption patterns to determine the contribution of diet to CAD development in black Africans. Furthermore there was no difference in physical activity (moderately active) nor was there any differences in socio-demographic profile as both groups came from the same urban environment. One possible contributing factor that was not measured in this study may be mental stress. Differences in coping skills, for example,

has been shown to increase inflammatory responses[40] and affect cardiometabolic risk[41] despite all participants having a similar socio demographic background. It is also possible that the use of questionnaires to determine self reported dietary intakes and physical activity were not sensitive enough to distinguish between subtle differences that may contribute to the development of CAD.

In conclusion, black South African CAD patients had increased levels of the same risk factors that are typically seen in Caucasians with insulin resistance and small dense LDL being particularly significant in their contribution. Only modest differences were observed between patients and controls for other risk factors. Apart from a lower vit C intake (possibly an indication of lower fruit and vegetable intake), no differences in dietary intakes and physical activity were observed between the CAD and control groups when matching for body fat, and both groups consumed more energy than required for a healthy body weight. It should be kept in mind that causality cannot be determined with a case control study design, and that generalisation of the study results should be done with caution due to the small sample size. Emerging data and our increasing understanding of the risk factor profile and pathophysiological processes involved in the increasing prevalence of CAD in black South Africans may have significant implications for the applicability of current public health dietary guidelines for the prevention of CAD in this group. Future research should be directed towards determining the cause of the insulin resistance and high prevalence of small dense LDL as possible strategies for preventing CAD in black South Africans.

ACKNOWLEDGEMENTS

We would like to thank the Circulatory Disorders Research Fund for funding this work. We would also like to thank the nursing staff at the Charlotte Maxeke Johannesburg Hospital as well as the patients for their willingness to be a part of this work.

REFERENCES

(1) Murray CJL, Lopez AD, Harvard School of Public Health, World Health Organization, World B. The global burden of disease a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020 ; summary. Cambridge, Mass.: Published by the Harvard School of Public Health on behalf of the World Health Organization and the World Bank; 1996.

(2) Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization. *Circulation* 2001;**104**:2746-53.

(3) Akinboboye O, Idris O, Akinboboye O, Akinkugbe O. Trends in coronary artery disease and associated risk factors in sub-Saharan Africans. *J Hum Hypertens* 2003;**17**:381-7.

(4) Sliwa K, Wilkinson D, Hansen C, et al. 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**:**9**15-22.

(5) Stewart S, Carrington M, Pretorius S, Methusi, P, Sliwa K. Standing at the crossroads between new and historically prevalent heart disease: effects of migration and socio-economic factors in the Heart of Soweto cohort study. *Eur Heart J* 2010; **32**(4):492-9.

(6) Vorster HH. The emergence of cardiovascular disease during urbanisation of Africans. *Public Health Nutr* 2002;**5**:239-43.

(7) Vorster HH, Venter CS, Wissing MP, Margetts BM. The nutrition and health transition in the North West Province of South Africa: a review of the THUSA (Transition and Health during Urbanisation of South Africans) study. *Public Health Nutr* 2005;**8**:480-90.

(8) Steyn K, Sliwa K, Hawken S, et al. Risk factors associated with myocardial infarction in Africa: the INTERHEART Africa study. *Circulation* 2005;**112**:3554-61.

(9) Steyn K, Fourie JM, Lombard CJ, Katzenellenbogen J, Bourne L, Jooste P. Hypertension in the black community of the Cape Peninsula, South Africa. *East African Medical Journal* 1996;**73**:758-63.

(10) Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet* 2005;**365**:217-23.

(11) Nethononda MR, Essop MR, Mbewu AD, Galpin JS. Coronary artery disease and risk factors in Black South Africans--a comparative study. *Ethn Dis* 2004;**14**:515-9.

(12) MacIntyre UE, Venter CS, Vorster HH. A culture-sensitive quantitative food frequency questionnaire used in an African population: 1. Development and reproducibility. *Public Health Nutr* 2001;**4**:53-62.

(13) Venter CS, MacIntyre UE, Vorster HH. The development and testing of a food portion photograph book for use in an African population. *J Hum Nutr Diet* 2000;**13**:205-18.

(14) Langenhoven ML, Kruger M, Gouws E, Faber M. MRC Food Composition Tables. 3rd ed. Parow: National Research Institute for Nutritional Disease of the South African Medical Research Council; 1991.

(15) Kruger HS, Venter CS, Steyn K. A standardised physical activity questionnaire for a population in transition: the THUSA study. *African journal for Physical, Health Education, Recreation and Dance* 2000;**6**:56-64.

(16) Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;**18**:499-502.

(17) Wing JR, Van der Merwe MT, Joffe BI, Panz VR, Seftel HC. Insulin-mediated glucose disposal in black south Africans with essential hypertension. *QJM* 1994;**87**:431-6.

(18) Holland Z, Ntyintyane L, Gill G, Raal F. Carotid intima-media thickness is a predictor of coronary artery disease in South African black patients. *Cardiovasc J Afr* 2009;**20**:237-9.

(19) Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001;**285**:2486-97.

(20) Bots ML, Grobbee DE. Intima media thickness as a surrogate marker for generalised atherosclerosis. *Cardiovasc Drugs Ther* 2002;**16**:341-51.

(21) Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med* 2006;**23**:469-80.

(22) Ntyintyane LM, Panz VR, Raal FJ, Gill GV. Metabolic syndrome, undiagnosed diabetes mellitus and insulin resistance are highly prevalent in urbanised South African blacks with coronary artery disease. *Cardiovasc J S Afr* 2006;**17**:50-5.

(23) Libby P. Inflammation and cardiovascular disease mechanisms. *Am J Clin Nutr* 2006;**83**:456S-60S.

(24) Tulenko TN, Sumner AE. The physiology of lipoproteins. *J Nucl Cardiol* 2002;**9**:638-49.

(25) Bisoendial RJ, Kastelein JJ, Stroes ES. C-reactive protein and atherogenesis: from fatty streak to clinical event. *Atherosclerosis* 2007;**195**:e10-e18.

(26) Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. *Arterioscler Thromb Vasc Biol* 2005;**25**:29-38.

(27) Schwartz EA, Reaven PD. Molecular and signaling mechanisms of atherosclerosis in insulin resistance. *Endocrinol Metab Clin North Am* 2006;**35**:525-49.

(28) Razani B, Chakravarthy MV, Semenkovich CF. Insulin resistance and atherosclerosis. *Endocrinol Metab Clin North Am* 2008;**37**:603-21.

(29) Niswender KD, Magnuson MA. Obesity and the beta cell: lessons from leptin. *J Clin Invest* 2007;**117**:2753-6.

(30) Rabe K, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. *Mol Med* 2008;**14**:741-51.

(31) Bjornheden T, Babyi A, Bondjers G, Wiklund O. Accumulation of lipoprotein fractions and subfractions in the arterial wall, determined in an in vitro perfusion system. *Atherosclerosis* 1996;**123**:43-56.

(32) Galeano NF, Al-Haideri M, Keyserman F, Rumsey SC, Deckelbaum RJ. Small dense low density lipoprotein has increased affinity for LDL receptor-independent cell surface binding sites: a potential mechanism for increased atherogenicity. *J Lipid Res* 1998;**39**:1263-73.

(33) Tribble DL, Rizzo M, Chait A, Lewis DM, Blanche PJ, Krauss RM. Enhanced oxidative susceptibility and reduced antioxidant content of metabolic precursors of small, dense low-density lipoproteins. *Am J Med* 2001;**110**:103-10.

(34) Rizzo M, Berneis K. Low-density lipoprotein size and cardiovascular risk assessment. *QJM* 2006;**99**:1-14.

(35) Shoji T, Hatsuda S, Tsuchikura S, et al. Small dense low-density lipoprotein cholesterol concentration and carotid atherosclerosis. *Atherosclerosis* 2009;**202**:582-8.

(36) Goff DC, Jr., D'Agostino RB, Jr., Haffner SM, Otvos JD. Insulin resistance and adiposity influence lipoprotein size and subclass concentrations. Results from the Insulin Resistance Atherosclerosis Study. *Metabolism* 2005;**54**:264-70.

(37) Garvey WT, Kwon S, Zheng D, et al. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. *Diabetes* 2003;**52**:453-62.

(38) Marais AD. Therapeutic modulation of low-density lipoprotein size. *Curr Opin Lipidol* 2000;**11**:597-602.

(39) Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation* 1990;**82**:495-506.

(40) Kop WJ, Weissman NJ, Zhu J, et al. Effects of acute mental stress and exercise on inflammatory markers in patients with coronary artery disease and healthy controls. *Am J Cardiol* 2008;**101**:767-73.

(41) Malan L, Malan NT, Wissing MP, Seedat YK. Coping with urbanization: A cardiometabolic risk? The THUSA study. *Biol Psychol* 2008;**79**:323-8.

(42) Lichtenstein AH, Appel LJ, Brands M, et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation* 2006;**114**:82-96.

(43) Van Horn L, McCoin M, Kris-Etherton PM, et al. The evidence for dietary prevention and treatment of cardiovascular disease. *J Am Diet Assoc* 2008;**108**:287-331.

(44) NICUS. Dietary Reference Intakes - Summarised from the six books on the Dietary Reference Intakes of the Institute of Medicine, Food and Nutrition Board, USA. Stellenbosch: Nutrition Information Centre, University of Stellenbosch; 2003.