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ANNEXURES

CORONARY ARTERY DISEASE IN BLACK SOUTH AFRICANS

Risk factor profile of coronary artery disease in black South Africans

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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.

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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]

Recognised risk factors that have been shown to be affected by urbanisation in the Transition and Health during Urbanisation in South Africans (THUSA) study include increases in hypertension, obesity, smoking habit and hyperfibrinogenaemia.[6] Changes in dietary intakes during urbanisation 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).

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. 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 three 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. A standardised and validated quantitative food frequency questionnaire, 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 programme, which is based on the South African Food composition tables.[14] A standardised Physical Activity questionnaire[15] 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 1 500g, for 20 minutes 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 1. 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 4.15mg/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 4.63mmol/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 ATP III 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).

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

TABLE 1: 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*

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 2 and compared to the dietary guidelines for the prevention of CAD. The only nutrient that was found to be significantly

TABLE 2: 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 a ctivity 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]
Polyunsaturated fatty acids % of TE	up to 10 % of TE[19]	6.96±2.17	7.84±2.37
Monounsaturated 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)	Minimise intake of foods and beverages with added sugars[42]	66.13[49.04 – 97.41]	63.77 [33.57 – 86.83]
Sodium (g)	Choose 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]* Female: 0 [0.00 – 0.00]	0 [0 – 2.63] Men: 1.21 [0.00 – 4.66]* Female: 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] Female: 39 [34.7 – 183]	61.3 [50.4 – 125]† Men: 64.4 [54.9 – 143] Female: 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: 1.7mg Female: 1.5mg[44]	1.38 [1.12 – 1.78] Men: 1.4 [1.12 – 1.78] Female: 1.34 [1.06 – 1.87]	1.55 [1.17 - 1.88] Men: 1.52 [1.29 – 1.68] Female: 1.57 [1.08 – 1.88]
B-Carotene (mg)	3 – 6mg[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.

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 27.5kg/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.

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 ß–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 triglycerides 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 vitamin 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, have 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 vitamin 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.

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ANNEXURE B: CONSENT FORM

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: …….

ANNEXURE C: SUBJECT QUESTIONNAIRE

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

<u>Dietary Assessment</u>

Date done: …….

By whom: ……..
<u>Examination</u>

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:……..
……
……..……..
……
……..

ANNEXURE D: QUANTIFIED FOOD FREQUENCY QUESTIONNAIRE

INSTRUCTIONS: Circle the subject's answer. Fill in the amount and times eaten in the appropriate columns.

I shall now ask you about the type and the amount of food you have been eating in the last few months. Please tell if you eat the food, how much you eat and how often you eat it. We shall start with maize meal porridge.

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
PORRIDGE AND BREAKFAST CEREALS AND OTHER STARCH								
Maize-meal porridge	Stiff (pap)						3400	
Maize-meal porridge	Soft (slappap)						3399	
Maize-meal porridge	Crumbly (phutu)						3401	
Ting								
Mabella	Stiff						3437	
Mabella	Soft							
Oats							3239	
Other cooked porridge	Type: _____							
Breakfast cereals	Brand name of cereals at home now: _____ _____ _____							

Do you pour milk on your porridge or cereal? Yes **1** No **2**

If yes, what type of milk (whole fresh, sour, 1%, fat free, milk blend, etc) _____

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
If yes, how much milk								
Do you put sugar on your porridge or cereal?		Yes **1**		No **2**				
If yes, how much sugar							3989	
							3989	
							3989	
Samp	Bought Self ground						3250	
Samp and beans	Give ratio of samp:beans						3402 (1:1)	
Samp and peanuts	Give ratio of samp:peanuts						3250 (samp)	
Rice	White						3247	
	Brown						3315	
	Maize Rice						3250	
Pasta	Macaroni Spaghetti Other specify: _____ _____						3262	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Pizza	Home made: Specify topping _____ _____						3353 (base+ ch)	
	Bought: Specify topping _____ _____						3353 (base+ ch)	
You are being very helpful. Can I now ask you about meat? **CHICKEN, MEAT, FISH** ***How many times do you eat meat (beef, mutton, pork, chicken, fish) per week?***_____								
Chicken (codes with skin)	Boiled						2926	
	Fried: in batter/crumbs Eg Kentucky						3018	
	Fried: Not coated							
	Bought: Chicken Licken						2925	
	Bought: Nando's							
	Roasted / Grilled						2925	
	Other: _____							
Do you eat chicken skin?	Always **1**		Sometimes **2**		Never **3**			
Chicken bones stew								
Chicken feet							2997	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Chicken offal								
Red meat	How do you like meat? With fat Fat trimmed							
Red meat	Fried							
	Stewed							
	Mince with tomato and onion						2987	
	Other:							
Beef Offal	Intestines: boiled nothing added						3003	
	Stewed with vegetables							
	Liver						2920	
	Kidney						2923	
	Other: Specify _____ _____							
Goat meat	Boiled						4281	
	Stewed with vegetables							
	Grilled / Roasted						4281	

What type of vegetables is usually put into meat stews?

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Wors / Sausage							2931	
Bacon							2906	
Cold meats	Polony						2919	
	Ham						2967	
	Vienna						2936	
	Other: Specify _____ _____							
Canned meat	Bully beef							
	Other: Specify _____ _____							
Meat pie	Beef						2939	
	Steak and kidney						2957	
	Cornish						2953	
	Chicken						2954	
	Other							
Hamburger	Bought							
Dried beans/peas/lentils	Soup						3145	
	Salad							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Soya products eg. Toppers	Brands at home now: _____ _____						3196 (Toppers)	
Pilchards in tomato/chilli/brine	Whole						3102	
	Mashed with fried onion							
Fried fish	With batter/crumbs							
	Without batter/crumbs							
Other canned fish	Tuna						3056 (oil)	
	Pickled fish							
	Other: Specify _____							
Fish cakes	Bought: Fried						3080	
	Home made with potato						3098	
Fish fingers	Bought						3081	
Eggs	Boiled/poached						2867	
	Scrambled: milk + fat							
	Fried: Fat							

Now we come to vegetables and fruit

VEGETABLES AND FRUIT

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Cabbage	How do you cook cabbage?							
	Boiled, nothing added						3756	
	Boiled with potato and onion and fat							
	Fried, nothing added Fried in ……							
	Boiled, then fried with potato, onion							
	Other:							
	Don't know							
Spinach/morogo/ beetroot leaves other green leafy	How do you cook spinach?							
	Boiled, nothing added						3913	
	Boiled with fat added Type of fat……...							
	With onion, tomato, potato							
	With peanuts							
	Other:							
	Don't know							
Tomato and onion gravy	Home made with fat Type of fat ……...							
	Without fat						3925	
	Canned						4192	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Pumpkin (yellow)	How do you cook pumpkin?							
	Boiled, nothing added						4164	
	Cooked in fat and sugar Fat ……							
	Boiled, little sugar and fat Fat ……							
	Other							
	Don't know							
Carrots	How do you cook carrots?							
	Boiled, nothing added						3757	
	Boiled, sugar and fat Fat ……							
	With potato and onion: Fat							
	Raw, salad						3709	
	Chakalaka							
	Other							
	Don't know							
Mealies/ **Sweet corn**	How do you eat mealies?							
	On cob – fat added Fat ……							

	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	On cob – no fat added						3725	
	Creamed sweet corn / canned						3726	
	Whole kernel/canned						3942	
Beetroot	Salad						3699	
	Boiled, nothing added						3698	
Potatoes	How do you cook potatoes?							
	Boiled/baked with skin						4155	
	Boiled/baked without skin						3737	
	Mashed							
	Roasted Fat ……							
	French fries (chips)						3740	
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked with skin						3748	
	Boiled/baked without skin						3903	
	Mashed							
	Other: _____							
	Don't know							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Salad vegetables	Mixed salad: tomato, lettuce and cucumber						3921	
	Raw tomato						3750	
	Other salad vegetables: _____ _____							
Other vegetables, specify + preparation	_____ _____ _____							
Do you like fruit?		Yes **1**	No **2**					
Apples							3592	
Pears							3582	
Oranges							3560	
Naartjie							3558	
Grapes							3550	
Peaches	Fresh						3565	
	Canned						3567	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Apricots	Fresh						3534	
	Canned						3535	
Mangoes							3556	
Guavas	Fresh						3551	
	Canned						3553	
Avocado							3656	
Wild fruit/berries	Specify type: _____							
Dried fruit	Types: _____							
Other fruit	_____ _____							
If subject eats canned fruit: Do you have custard with the canned fruit? Yes **1** No **2**								
Custard	Home made: Milk							
	Commercial eg Ultramel						2716	
BREAD AND BREAD SPREADS								
Bread / Bread rolls	White						3210	
	Brown						3211	
	Whole wheat						3212	
Do you spread anything on the bread? Always **1** Sometimes **2** Never **3**								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Margarine	What brand do you have at home now?							
	Don't know _____							
Peanut butter							3485	
Jam/syrup/honey							3985	
Marmite / Fray bentos / Oxo							4058	
Fish/meat paste							3109	
Cheese	Type: _____ _____ _____							
Achaar								
Other spreads	Specify: _____ _____							
Dumpling								
Vetkoek	White flour						3257	
	Whole wheat flour						3324	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Provita, crackers, etc							3235	
Mayonnaise / salad dressing	Mayonnaise						3488	
	Other: Specify _____							
DRINKS								
Tea	English (normal)						4038	
	Rooibos						4054	
Coffee							4037	
Sugar/cup tea or coffee	Tea:						3989	
	Coffee:						3989	
Milk/cup tea or coffee	What type of milk do you use in tea and coffee?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Whole milk powder Brand: _____						2721 (powder)	
	Low fat milk powder Brand: _____						2825 (powder)	

	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Skimmed milk powder Brand: _____						2825 (powder)	
	Milk blend Brand: _____						2770 (powder)	
	Whitener: type _____ _____							
	Condensed milk						2714	
	Evaporated milk						2715	
	None							
Milk as such	What type of milk do you drink milk as such?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Condensed milk						2714	
	Sour/maas						2787	
	Other: _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Milk drinks	Nestle: _____							
	Milo: _____							
	Flavoured milk: _____							
	Other:							
Yoghurt	Drinking yoghurt						2756	
	Thick yoghurt						2734	
	Low fat sweetened with fruit						2732	
Squash	Sweet O						4027	
	Six O							
	Oros/Lecol – with sugar						3982	
	- artificially sweetener						3990	
	KoolAid						4027	
	Other: _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Fruit juice	Fresh/Liquifruit/Ceres						2866	
	Tropica (Dairy –fruit juice mix)						2791	
	Other: _____ _____ _____							
Fizzy drinks **Coke, fanta, etc**	Sweetened						3981	
	Diet							
Maueu/Motogo							4056	
Home brew								
Tlokwe							4039	
Beer							4031	
Spirits							4035	
Wine red							4033	
Wine White							4033	
Other specify	_____ _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
SNACKS AND SWEETS								
Potato crisps							3417	
Peanuts	Raw						4285	
	Roasted						3458	
Cheese curls, Niknaks, etc							3267	
Raisins							3552	
Peanuts and raisins								
Chocolates	Name: _____ _____ _____							
Candies	Sugus, gums, hard sweets, etc						4000	
Sweets	Toffees, fudge, caramels						3991	
Biscuits/cookies	Type: _____ _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Cakes and tarts	Type: _____ _____ _____							
Scones								
Rusks	Type: _____ _____							
Savouries	Sausage rolls						2939	
	Samoosas: Meat filling						3355	
	Samoosas: Vegetable filling						3414	
	Biscuits eg bacon kips							
	Other specify: _____ _____							
Jelly							3983	
Baked pudding	Type: _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Instant pudding	Milk type: _____							
Ice cream							3483	
Sorbet							3491	
Other specify	_____ _____ _____							
SAUCES, GRAVIES AND CONDIMENTS								
Tomato sauce / Worcester sauce							3139	
Chutney							3168	
Pickles							3866	
Packet soups							3165	
Other:	_____ _____							
WILD BIRDS, ANIMALS OR INCECTS (hunted in rural areas or on farms)								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Wild fruit								
MISCELLANEOUS: Please mention any other foods used more than once/two times a week which we have talked about:								
INDIGENOUS/TRADITIONAL FOODS/PLANTS/ANIMALS Please tell me if you use any indigenous plants OR other indigenous foods like mopani worms, locusts ect to eat								
Specify								

ANNEXURE E: PHYSICAL ACTIVITY QUESTIONNAIRE

Physical activity questionnaire

Date: Place: Interviewer:

The information on this questionnaire is confidential

1.	Subject number						(1-4)
2.	Gender	Male	1	Female	2		(5)
3.	What is your main occupation?.....						
	Low level: office work, housework, scholar				1		
	Middle level: factory work, carpentry, farming, hospital nurse, plumber				2		(6)
	High level ("sweat work"): construction work, digging, manual labour				3		
4.	At work I sit	1. never	2. seldom	3. sometimes	4.often	5.always	(7)
5.	At work I stand	1. never	2. seldom	3. sometimes	4.often	5.always	(8)
6.	At work I walk	1. never	2. seldom	3. sometimes	4.often	5.always	(9)
7.	At work I lift heavy loads	1. never	2. seldom	3. sometimes	4.often	5.always	(10)
8.	At work I am tired	1.never	2.seldom	3. sometimes	4.often	5.always	(11)
9.	At work I sweat	1.never	2.seldom	3. sometimes	4.often	5.always	(12)
10.	If you work away from home, how do you get to work/school?				walk	1	
					cycle	2	(13)
					car/taxi	3	
11.	How long does it take you to walk/cycle to work/school? (or to the taxi rank/ bus stop/ train station)				0-15 min	1	
					16-30 min	2	
					31-60 min	3	
					1-2 hours	4	(14)
12.	If you walk or cycle to work/school, what is your usual pace? (or to taxi rank/bus stop/ train station)				casual strolling	1	
					fairly brisk	2	
					brisk/fast	3	(15)
13.	Do you climb stairs often?				yes	1	
					no	2	(16)
14.	If yes, how many flights of stairs do you climb each day? (1 flight = 10 steps)						(17)
15.	How many days per week do you climb steps?						(18)
16.	Do you play sport?				yes	1	
					no	2	(19)
17.	Which sport do you play most frequently?	low level: bowling, golf, billiards				1	0.76*[1]
		middle level: tennis, athletics, cycling				2	1.26
		high level: soccer, rugby, netball, boxing				3	1.76(20)
		If other, specify					
18.	How many hours per week do you practice?<1/ 1-2/ 2-3/ 3-4/ >4 (Write appropriate code in space) 0.5, 1.5, 2.5, 3.5, 4.5*[2]						(21-23)
19.	How many months per year ? (Write appropriate code in space) <1/ 1-3/ 4-6/ 7-9/ >9 0.04, 0.17, 0.42, 0.67, 0.92*[3]						(24-26)

*[1] intensity code of sport, *[2] time code for sport, *[3] proportion of year

20.	If you play a second sport, which is it?	low level: bowling, golf, billiards					1	0.76*[1]
		middle level: tennis, athletics, cycling					2	1.26
		high level: soccer, rugby, netball, boxing					3	1.76(27)
		Other, specify						
21.	How many hours per week do you practice?	<1/ 1-2/ 2-3/ 3-4/ >4 0.5, 1.5, 2.5, 3.5, 4.5*[2]					.	(28-30)
22.	How many months per year?	<1/ 1-3/ 4-6/ 7-9/ >9 0.04, 0.17, 0.42, 0.67, 0.92*[3]					.	(31-33)
23.	During leisure time I watch TV/ do sitting activities (read, study, play cards)	1.never	2.sel-dom	3.some-times	4.often	5.al-ways		(34)
24.	During leisure time I walk/ do standing activities (gardening, housework)	1.never	2.sel-dom	3.some-times	4.often	5.al-ways		(35)
25.	Other leisure-time activities:..... (leisure-time = time off from work/. school)		2.sel-dom	3.some-times	4.often	5.al-ways		(36)

Definitions and explanation of the questionnaire (interviewer's notes)

Item 1: Write in the subject number as on the name label provided at the recruitment station.
Item 2: Circle gender: male or female
Item 3. Occupation: paid job or unpaid duties for most of the day; including school, housework, childminding
Write in the occupation stated and circle 1,2 or 3 (low level, middle level or high level)

Item 4-9:	never:	⊕:	never, almost never
	seldom:	⊕	one-quarter of the workday or workweek
	sometimes:	⊕	half the workday or workweek
	often:	⊕	three-quarters of the workday or workweek
	always:	⊕	almost all the time

Item 13. If the subject does not climb stairs, go on to question 16.
Item 16: If the subject does not play sport, go on to question 23.
Item 17: Circle 1/2/3
Item 18 and 21: Write time code in space, note decimal point
Item 19 and 22: Write code in space, note decimal point
Item 20: Circle 1/2/3

Item 23-25:	never:	⊕	never, almost never
	seldom:	⊕	one-quarter of off-time, 1-2 days per week
	sometimes:	⊕	half my off-time, 3-4 days per week
	often:	⊕	three-quarters of my off-time, 5-6 days per week
	always:	⊕	almost all the time, mostly 7 days per week

Item 23: sitting activities: watch TV, listen radio, reading, writing, knitting, needlework, playing cards, visiting friends
Item 24: standing activities: gardening, walking with friends, cleaning, cooking, doing laundry, ironing, dishwashing after work at your own home
Item 25: other leisure-time activities: name any other leisure-time activities that you do and how often you do these activities.
NB: *leisure-time* is time after work, school, or housework is finished

Calculate the work-index, items 3-9: $[I_3 + (6-I_4)^* + I_5 + I_6 + I_7 + I_8 + I_9]/7$

Sum of all items` scores (maximum 5) divided by 7;

* Item 4 reversed because highest score for lowest activity level

Calculate the commuting-index, items 10-12: 0 for people who do not commute

$[(4 - I_{10}) + I_{11} + I_{12}]/3$

Calculate the stairs-index: 0 for people who do not climb stairs

$= I_{14} \times I_{15}/7$

Calculate the sport-scores (I_{16} and I_{20}, 0 for people who do not play sport)

= [intensity x time x proportion of year]; Sport index = $I_{16} + I_{20}$

Calculate the leisure-time index: $[(6 - I_{23}) + I_{24} + I_{25}]/3$

Composite physical activity (PA)-index

= Work-index + commuting-index + stairs-index + sport index + leisure-time-index

Calculate a weighted composite PAI for proportionate time spent in each activity category:

=0.47(work-index) + 0.059(commuting index) + 0.001(stairs-index) + 0.47(sport index + leisure-time-index)

Factors for the weighted index may be changed for a study population for which times spent in main occupation and leisure-time differs much from the proportions stated here.

ANNEXURE F: PURE-SA INFORMED CONSENT FORM

POTCHEFSTROOM CAMPUS

PURE-SA Project (Prospective Urban and Rural Epidemiology)

INFORMED CONSENT FORM (including the PRIMER-study)

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

I agree to be tested for HIV	Yes	No
I want to know my HIV-status	Yes	No
I agree to give a blood sample	Yes	No

I hereby also declare that I am aware that:

1. this blood sample will be used for the purpose of
   a. Isolating DNA to look at genetic factors that are currently associated with Type 2 Diabetes (i.e. the Calpain10, Adiponectin, Leptin and Leptin Receptor genes), or genetic factors that may be associated with Non Communicable diseases in the future. We give the assurance that all genetic tests and experiments will only focus on genotypes suspected to contribute to an increased risk of non communicable diseases of lifestyle.
   b. Testing for liver function by determining liver enzymes such as AST, GGT,
   c. Analyses of other than genetic parameters for Diabetes Mellitus such as HbA_1C, Blood glucose and Insulin
   d. Analyses of clotting factors and hypertension markers
   e. Analyses of bone health, iron and nutrition status
   f. And may be stored until such time as the above measurements/analyses will be done.
2. A two hour glucose tolerance test will be done
3. Body measurements such as height, weight, skinfold thicknesses, arm and leg circumferences will be taken
4. Electrocardiograph be taken
5. Blood pressure to be taken
6. Pulse wave velocity measurements will be made
7. A urine sample to be collected to analyse for the presence of heavy metals such as lead and mercury,
8. A Spirometer test to be performed to determine lung function
9. A handgrip test to be performed to test muscle strength
10. A hair sample to be taken to test for fumonisin mycotoxins.

.....
(Signature of the subject)
Signed at ... **Potchefstroom / Ganyesa** ... (delete not applicable option) on ...../...../ **2005**

Witnesses

1. ..... 2. .....

Signed at ... **Potchefstroom / Ganyesa** ... (delete not applicable option) on ...../...../ **2005**

PART 1

1. **School/Institute:**
Faculty of Health Sciences, North-West University

2. **Title of project/trial:**
PURE: Prospective Urban and Rural Epidemiological study

3. **Full names, surname and qualifications of project leader:**
Dr. Annamarie Kruger, Ph.D. (Nutrition)

4. **Rank/position of project leader:**
Research Manager

5.. **Aim of this project**

PURE's aim is that understanding the different lifestyle and health transitions of individuals in response to societal changes will elucidate societal and individual adaptive strategies that could diminish the adverse health effects of industrialization and urbanization on health, while retaining its benefits.

6. **Explanation of the nature of all procedures, including identification of new procedures:**

Each participant will have to fill in a number of questionnaires (Adult questionnaire, Physical activity questionnaire, Food frequency questionnaire, Health questionnaire) with the help of field workers. A blood and urine sample will be taken. Physical measures will be performed, including anthropometric measures (such as weight, height, and waist circumference), blood pressure, lung capacity and lung volume and an ECG will be performed.

7. **Description of the nature of discomfort or hazards of probable permanent consequences for the subjects which may be associated with the project: (Including possible side-effects of and interactions between drugs or radio-active isotopes which may be used.)**

It will take each participant quite a while (about two hours) to complete all the tests and discomfort may be experienced with the taking of blood samples. No measures will have permanent damage or consequences for the participants.

8. **Precautions taken to protect the subjects:**

The research nurse will be present at all times, and will be responsible for the blood sampling. She is very experienced and has performed these procedures numerous times in previous studies.

9. **Description of the benefits which may be expected from this project:**

When measures with immediate results are taken, such as blood glucose levels or blood pressure, the information will be communicated to the individual to seek professional help. Since this study is a longitudinal study, subjects that are high at risk will be identified from the dataset and personal feedback will be given.

10. **Alternative procedures which may be beneficial to the subjects:**

There will be tested for HIV/AIDS, therefore pre-test counselling will be given. If the subject wants to know his/her status and he/her tests positive, post counselling will also be given.

PART 2

To the subject signing the consent:
You are invited to participate in a research project. It is important that you read/listen to and understand the following general principles, which apply to all participants in our research project:

1. **Participation in this project is voluntary.**

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

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

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

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

6. **The University staff will use standardised procedures and take all possible precaution to protect the subject from risks.**

7. **All information will be kept CONFIDENTIAL and no personal information will be published without my consent.**

Dr ANNAMARIE KRUGER

Contact details: 082 771 5778 / 018 299 4037(Office)