CARDIOVASCULAR RISK FACTORS AMONG 15-20 YEARS OLD RURAL SUBJECTS RESIDING IN DIKGALE DEMOGRAPHIC SURVEILLANCE SITE (DDSS), LIMPOPO PROVINCE

by

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DISSERTATION

Submitted in fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

MEDICAL SCIENCES

(Chemical Pathology)

in the

Faculty of Health Sciences

(School of Health Sciences)

at the

UNIVERSITY OF LIMPOPO

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2013

DECLARATION

I, Nkosinathi Terrence Phoku, declare that the work for this dissertation, unless where acknowledged, is written, compiled and produced by me. This work has not been submitted anywhere else for completion of any academic qualification.

Signature: _____ Date: _____

DEDICATION

I dedicate this project (Cardiovascular risk factors among 15-20 years old rural subjects residing in Dikgale Demographic Surveillance Site (DDSS), Limpopo Province) to my family, extended family and my future family.

ACKNOWLEDGEMENT

This project should not have been successful without the help of the following people:

- Prof Alberts M for supervising this project
- Mr Choma SSR for academic, technical assistance and co-supervision
- Nurses for helping in blood collection
- Residents in DDSS area for consenting and donating blood to the study
- My co-project mates for team work
- God, Almighty for taking me this far
- Medical Research Council (MRC) for funding me in this project

Presentation at Conferences

1. Academic day, University of Limpopo (Medunsa Campus).

Title: The Association between apoB and LDL-C and between apoA-1 and HDL-C in a South African rural black population.

Date: 28 August 2008

Type: Oral presentation

ABSTRACT

Cardiovascular diseases (CVDs) are among one of the well documented conditions and pose a significant health burden in the world as they areconsidered to be of adult onset.However, recent studies have shown that in developed countries CVD risk factors are becoming prevalent in young people which isof great concern. Therefore, the aim of this study was to determine if CVD risk factors are present in young subjects aged 15-20 years of age residing in a rural area of a developing country.

Methods:
Subjects aged between 15-20 years who participated in the ñGene - Environment interaction projectò were included in this sub-study. Total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, insulin, glucose, creatinine, Lp(a), apoB, apoA-1 and hs-CRPwere determined. Blood pressure, physical activity (number of steps/day), weight, height, waist circumference and hip circumference were obtained from the database. Subjects with CRP levels above 10mg/L and creatinine levels above 130 mmol/L were excluded.

Results:
The present study showed an overall high prevalence of some CVD risk factors. There was high prevalence of insulin resistance (23.0% in females and 34.7% in males), and high hs-CRP (18.4% in females, 12.9% in males). The prevalence of low HDL-C levels was high (55.2% in females and 16.8 % in males), however, the prevalence of abnormal levels of other lipids such as total cholesterol/HDL-cholesterol ratio was low in both males and females. The prevalence of an increased apoB/apoA ratio was significantly higher in females 26.4% compared to males 7.9%. The prevalence of overweight (12.6%) and obesity (9.2%) was higher in females than in males (overweight 1%, obesity 0 %). The prevalence of hypertension was comparable between the two genders (5.7% in females and 10.9 % in males).

Conclusion:
The results showed a relatively high prevalence of non-traditional risk factors for cardiovascular diseases in adolescents residing in a rural area, Limpopo Province, while the prevalence of traditional risk factors such as total cholesterol and triglycerides was low.

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1. Introduction

Cardiovascular diseases (CVDs) are a well-documented and significant health burden in the world [1]. Clinical manifestation of CVDs is apparent in adults [2], but several studies have shown that atherosclerosis is a process that begins in childhood [3, 4]. Risk factors for CVD, such as hypertension, diabetes mellitus, obesity, insulin resistance, hypercholesterolemia, low physical fitness, cigarette smoking and physical inactivity are not only present in adults but are also becoming prevalent in young people [5, 6, 7].

Previously CVD risk factors were rare among South African black populations [8] but more recent studies have reported the emergence of CVD risk factors in black subjects and the lipid profile shows signs of a change towards that of the westernized population [9, 10]. Badenhorst *et al*. (2004), reported that environmental factors associated with rapid urbanization including psycho-economic factors and adoption of a westernized diet accompanied by a more sedentary lifestyle are important factors in the pathogenesis of cardiovascular disease in developing countries [11].

Several studies in both developed and developing countries have reported that overweight and obesity are appearing in progressively younger subjects both in rural and urban areas [12, 13] and as a result other CVD risk factors such as hypertension, dyslipidaemia, impaired glucose tolerance and vascular abnormalities are being observed in children and adolescents [12,14].

Cardiovascular diseases risk factors are also increasing in adults residing in rural areas of South Africa due to changes in lifestyle and high prevalences of obesity, hypertension, type 2 diabetes mellitus and dyslipidaemia were reported in this study population [15]. Therefore, a decision was taken to determine if CVD risk factors were also present in adolescents residing in this rural area, situated in Limpopo Province, Republic of South Africa.

1.2 Hypothesis

A high prevalence of CVD risk factors is present in rural subjects aged 15-20 years of age residing in Dikgale Demographic Surveillance Site (DDSS).

1.3 Aim

To determine the prevalence of CVD risk factors in young rural subjects aged 15-20 years of age residing in DDSS.

1.4 Objectives

- To measure anthropometric parameters (weight, height, waist circumference, hip circumference) in subjects.
- To determine serum insulin levels and plasma glucose concentrations.
- To determine serum lipids levels of HDL-C, LDL-C, Trig, TC, Lp(a), apoA and apoB.
- To determine blood pressure (SBP, DBP).
- To determine serum hsCRP levels in subjects.
- To determine physical activity, alcohol and tobacco use by subjects.

2 Literature review

Cardiovascular diseases has a multifactorial aetiology. Obesity [16], hypertension [17], insulin resistance [18], diabetes mellitus [19], inflammation [20] and dyslipidaemia [21] have been individually associated with development of CVD. It has also been shown that interactions between different risk factors rather than an individual risk factor alone seem to be more important in the development of CVD [22, 23].

Metabolic syndrome is a cluster of more than three risk factors for CVD which are hypertension, obesity, low HDL cholesterol, increased triglycerides, increase glucose and hyperinsulineamia [24]. Metabolic syndrome has been reported to be associated with an increased risk of developing CVD [24, 25, 26]. Metabolic syndrome has been defined differently by the International Diabetes Federation (IDF), refer to table 2.1 [27] and the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III), refer to table 2.2 [28].

Table 2.1: Metabolic syndrome definition by IDF

Adult Metabolic syndrome definition	Adolescents metabolic syndrome definition
Three or more of the following: 1. Fasting glucose Ó5.6 mmol/L 2. Waist circumference 94cm (males) and 80cm (females) 3. Triglycerides Ó 1.7 mmol/L 4. HDL-C < 1.03 mmol/L (males) and <1.29 (Females) 5. SBP Ó130 or DBP Ó85 mmHg	Three or more of the following: 1. Fasting glucose Ó5.6 mmol/L 2. Waist circumference Ó 90^{th} percentile (age and sex specific) 3. Triglycerides Ó 1.7 mmol/L 4. HDL-C < 1.03 mmol/L 5. Blood pressure > 90^{th} percentile

Table 2.2:Metabolic syndrome definition by NCEP-ATP III

Adult Metabolic syndrome definition	Adolescents metabolic syndrome definition
Three or more of the following: 1. Central obesity > 102cm (male), >88cm (female) 2. Triglyceride Ó1.7 mmol/L 3. HDL-C < 1.0 mmol/L (male), <1.2 mmol/L (female) 4. Blood pressure Ó 135/85 mm Hg or medication 5. Fasting glucose Ó6.1 mmol/L	1. Central obesity Ó 95th percentile (age and sex specific) 2. Triglyceride Ó1.7 mmol/L 3. HDL-C < 1.0 mmol/L (male), <1.3 mmol/L (female) 4. Blood pressure Ó 95th percentile (age and sex specific) 5. Fasting glucose Ó6.1 mmol/L

In a study by Mollentze *et al*. (1995), at least one risk factor (hypercholesterolaemia, hypertriglyceridaemia, low HDL cholesterol, hypertension, obesity or diabetes) for CVD occurred as frequently among black subjects as in white subjects, although CVD like ischemic heart disease has thus far remained rare in the South African black population [29]. These authors also stated that the small racial difference in HDL-C levels alone does not explain the rarity of CVD in the black population. Furthermore, the study did not find any difference in risk factors between urban and rural populations. Thus they concluded that the processes associated with urbanization are now no longer restricted to the urban areas but also occur in rural settings [29].

The risk factors associated with CVD that will be discussed below are dyslipidaemia [30], inflammation [31, 32], type 2 diabetes [33], insulin resistance [34], blood pressure [35], obesity [15], physical inactivity [36], stunting [37], smoking [38] and alcohol intake [39].

2.1 Serum lipids

Organisms use lipoproteins to move hydrophobic lipid molecules through the aqueous blood and tissue lymph for uptake by peripheral cells [40]. Lipoprotein particles are defined by their complement of associated apolipoproteins and by their cholesterol, triglyceride and phospholipid

content. The protein and lipid content defines the particle buoyant density and divides them into major classes [40]:

a) High density lipoproteins (HDL)
b) Low density lipoproteins (LDL)
c) Very low density lipoproteins (VLDL)
d) Intermediate density lipoproteins (IDL)
e) Chylomicrons

Lipoproteins have apolipoproteins which are small proteins found on the surface of a lipoprotein, where they play an important role in maintaining the structural integrity and functional specificity of plasma lipoproteins [41]. Apolipoproteins also perform various functions in the metabolism of lipoproteins, including their secretion, retardation of their premature removal, recognition of their binding and removal sites on cellular surfaces and activation of lipolytic enzymes [41, 42].

The clinical uniqueness of apolipoproteins makes them useful as specific markers of identifying and classifying discrete lipoprotein particles, irrespective of their density or other non-specific physical properties [41]. Table 2.3; shows the different Lipoprotein class and their unique clinical characteristics (density, size, major lipids and major apolipoproteins) that were characterized by Tulenko and Summer (2002) [43].

Table 2.3: Differential lipoprotein characteristics

Lipoprotein class	Density(g/mL)	Size (nm)	Major lipids	Major apolipoproteins
Chylomicron	0.93	100-500	Dietary Trigs	B-48,C-II, E
VLDL	0.93-1.006	30-80	Endogenous Trigs	B-100, C-II, E
IDL	1.006-1.019	25-50	CEs and Trigs	B-100, E
LDL	1.019-1.063	18-28	CEs	B-100
HDL	1.063-1.210	5-15	CEs	A-1, C-II, E

2.1.1 Lipoprotein metabolism

Forward cholesterol transport is the transfer of both exogenous and endogenous cholesterol from the liver to the peripheral cells [43]. Chylomicrons are lipoprotein particles that are synthesized in the intestinal mucosal cells directly from dietary fats, namely triglycerides, cholesterol and phospholipids, and apoprotein (Apo) B-48, which is also synthesized in these cells [43, 44]. Their density is low because their protein content is low, and they contain large amounts of lipid (especially buoyant triglycerides). Their large size precludes penetration of the capillary membrane, so they are secreted by the intestinal mucosa into the lymphatics and then enter the circulation by way of the thoracic duct [43, 45].

Once in the blood, chylomicrons acquire apo E and apo C-II from HDL and other lipoprotein remnant particles and are progressively reduced in size by the action of lipoprotein lipase, which is bound to the capillary endothelium and catalyzes the removal of free fatty acids from the chylomicron triglyceride pool [43, 46, 47]. Lipoprotein lipase is activated by apo C-II on the chylomicron, and proceeds to hydrolyze most of the chylomicron triglycerides to free fatty acids, which are then released from the particle and bind to albumin, and are ultimately deposited in adipose tissue [43].

The continued action of lipoprotein lipase leaves the chylomicron nearly depleted of triglyceride within an hour after a meal [43, 46, 47]. The depleted chylomicron remnant particle is removed by its uptake into the hepatocytethrough a receptor-mediated process, with the chylomicron apo E serving as the ligand for the hepatic LDL receptor [42, 46, 47]. In the hepatocyte the chylomicron remnant releases its content of remaining triglycerides, cholesteryl esters, phospholipids, and apolipoproteins. The hepatocyte reassembles these chylomicron remnantï derived products, along with endogenous triglycerides and cholesteryl esters, into very low-density lipoproteins (VLDLs) and secrete them into the circulation [43, 46, 47].

Lipoprotein lipase (activated again by apo C-II on the VLDL particle) reduces VLDL triglyceride content further, leaving the particles progressively smaller, denser, and more cholesterol-enriched: these are known as Intermediate Density Lipoprotein (IDL) [43, 48].

Hydrolysis of IDL triglycerides to free fatty acids is mediated by hepatic lipase and LDL is the terminal particle in this pathway. About one third of the VLDL is cleared by the hepatic LDL receptor, whereas two thirds of the VLDL undergoes reduction of triglyceride content resulting in IDL and subsequent hydrolysis of the IDL triglycerides to fatty acids terminating as LDL. The transition of VLDL to IDL is accompanied by the transfer of apo C-II to HDL while the transition of IDL to LDL is accompanied by the transfer of apo E to HDL, resulting in a LDL bound to apo B-100 [43, 49]. Cholesterol rich LDL particles are then taken up by peripheral tissues in an endocytotic process that involves the LDL receptor through apolipoprotein B [43].

The metabolic balance of cholesterol is accomplished by reverse cholesterol transport by HDLs.HDL is formed by the transfer of cholesterol and phospholipids onto apolipoproteinA-1 (ApoA-1) to generate preɓ-HDL [40, 43, 50]. This process is catalyzed by the ATP-binding cassette A1 (ABCA1) transporter, which is expressed in the peripheral tissues, intestine and liver. The cholesterol in the nascent pre ɓ-HDL is then esterified by lysolecithin cholesterol acyltransferase (LCAT) as part of a process that generates mature spherical HDL. The ATP-binding cassette GI(ABCG1), another ABC transporter, is able to load more cholesterol onto mature HDL from peripheral tissues and along with ABCA1 is important in allowing macrophages to remove artery wall cholesterol, which prevents atherosclerosis. The HDL cholesterol-esters are taken up by scavenger receptor BI (SRBI) in the liver and after hydrolysis the resulting free cholesterol is metabolized to bile acids (BA) and excreted from the body in the faeces [40, 43, 50].

Dietary cholesterol is also packaged into HDL particles by the action of ABCA1 and ABCG1, and as HDL circulates there is an increase in its apoCII and apoE ratio because of a protein exchange between HDL and VLDL [40, 43, 51]. Cholesterol esters from HDL are also transferred to VLDL remnant particles (IDL) by the action of cholesterol ester transfer protein (CETP). Intermediate density lipoprotein (IDL) loses most of apolipoprotein except apoB and is converted to LDL by the action of hepatic lipase. Finally, LDL is taken up by the liver and other tissues in an endocytotic process that involves the LDL receptor [40, 43, 51].

2.1.2 Dyslipidaemia

Dyslipidaemia is defined as an increase in either triglycerides, cholesterol, LDL-C or a decrease in HDL-C. Dyslipidaemia has been reported as the main risk factor in the development of atherosclerosis which results in cardiovascular events [52].

Total serum cholesterol is the cholesterol found in IDL, LDL, VLDL and HDL particles and blood total cholesterol levels greater than 5.00 mmol/lare regarded as dyslipidaemic [53].Low Density Lipoprotein cholesterol(LDL-C) is the cholesterol found in the atherogenic LDL particle and blood Low Density Lipoprotein cholesterol (LDL-C) levels greater than 3.00 mmol/l are regarded as dylipidaemic [54]. High Density Lipoprotein cholesterol (HDL-C) is the cholesterol found in the anti-atherogenic HDL particle and a blood level of less than 1.00 mmol/l in males and less than 1.20 mmol/l in females is regarded as dylipidaemic [55]. Blood triglyceride levels of Ó1.70 mmol/lare regarded as dylipidaemic [54].

There are several ways in which dyslipidaemia can result in CVD. One of the mechanisms is LDL mediated. Low density lipoprotein particle elevations together with low HDL- particle levels are regarded as risk factors in the pathogenesis of CVD [56]. The increase in the LDL particle levels in the arterial wall increases the chances of the LDL particle modification by oxidation [56]. Low density lipoprotein particle oxidation can result in the production of free radicals which damages both the lipid and protein components of the LDL particle and furthermore, can damage an unsaturated fatty acid side chain, generating more free radicals and leading to more oxidative damage and rancidity in the core of the particle [21, 56]. This process may also damage the apolipoproteins, causing both fragmentation and aggregation resulting in foam cell formation in the arterial wall. Oxidized LDL particle is cytotoxic to a variety of vascular cells and result in stimulation of plaque formation thus leading to atherosclerosis which eventually leads to cardiovascular events [21].

Another mechanism is LDL receptor mediated. An increase in the apoB-containing lipoproteins may result in apoB receptor saturation which may lead to increased apoB-containing lipoproteins in the circulation and result in increased cholesterol intake by macrophages [21]. The increased

cholesterol intake by macrophages will result in foam cell formation that causes plaque formation resulting in CVD events [21].

Abnormal lipid levels are related to unhealthy dietary habits and lifestyle during childhood and adolescence [12] and has also been shown to also be genetically inherited [57]. Dyslipidaemia shows higher prevalence in children with obesity compared to non-obese children [58, 59]. A high prevalence of hypercholesteroleamia, hypertriglycerideamia and low HDL-C was reported in 13 year old Mexican obese adolescents [12].

Recently there is increasing evidence that apolipoproteinB (apoB) and apolipoproteinA-1 (apoA-1) are better indicators of serum lipid abnormalities than LDL-C/HDL-C ratio as indicators of serum lipid abnormalities[60, 61, 62].Circulating levels of apolipoproteins reflect the number of lipid particles. The level of apoB reflects the number of triglyceride rich VLDL particles and the number of LDL particles [29]. ApolipoproteinB concentration gives more weight to the number of small dense LDL particles than the more regular measurement of LDL-C [63].The level of apolipoprotein A-1 reflects the number of HDL particles [64]. Plasma levels of apoA-1, the major apolipoprotein in HDL-C, have been negatively correlated with CVD while the plasma levels of apoB the major apolipoprotein in LDL-C have been positively correlated with CVD [65].

Thus the ratio of apoB/apoA-1 would, therefore, theoretically be an ideal marker for lipoprotein disturbances [63]. The apoB/apoA-I ratio is said to indicate more accurately CVD risk than LDL-C and other lipids levels [66]. A study done by Walldius *et al*. (2004), reported that irrespective of the lipid phenotypes, the greater risk of dying from acute myocardial infarction was found in subjects having a high apoB/apoA-1 ratio compared with those having a low ratio [61]. The higher the value of the apoB/apoA-I ratio, the more cholesterol and triglyceride circulating in the plasma provoking atherogenesis [66]. A low apoA-I component of the ratio also reflects a low HDL-C, which occurs together with high triglyceride levels in patients having small dense-LDL particles [66].

Abnormal lipid levels are not only related to unhealthy dietary habits and lifestyle factors but also to genetic factors [57]. Familial hypercholesterolaemia II (FH II) has been reported as one of the important risk factors in the development of atherosclerosis and CVD [57, 67]. Familial hypercholesterolemia II is an inherited disorder characterized by a high concentration of serum total cholesterol and LDL cholesterol. This inherited disorder results from different mutations in the gene coding for the LDL-receptor thus causing a defective LDL-receptor function which results in high serum LDL cholesterol [58]. The high LDL cholesterol levels frequently give rise to xanthomas, increase deposit of LDL cholesterol in peripheral tissues and accelerated atherosclerosis resulting from cholesterol deposition in the arterial wall thus causing atheromatous plaque, thereby increasing the risk of CVD [68, 69].

2.1.3 Lipoprotein (a)

Lipoprotein (a) (Lp(a)) is the assembly of LDL particles and a carbohydrate ï rich protein called apolipoprotein(a) [70]. The apoB100 and apo(a) are linked together by a disulfide bridge [70]. The lipid particle that has a smaller size apo(a) is confined to the low density Lp(a) species, whereas theapoB100 complex containing a larger size apo(a) occurs in the high density Lp(a) species [71]. Moderate levels of Lp(a) were reported to be significantly higher in individuals with high LDL-C levels than those with normal LDL-C [72]. Individuals with moderate and high total cholesterol levels had significantly higher mean Lp(a) levels compared to individuals with normal total cholesterol [72], the association between serum Lp(a) and LDL-C or total cholesterol have also been noted in other studies [73, 74]. A study by Obisesan *et al*. (2004), reported an association between serum Lp(a) and LDL-C or total cholesterol in children and adolescents of different ethnic groups (African American, non-hispanic White and Mexican American) [75].

It has been shown that considerable heterogeneity of the Lp(a) polymorphism exists among populations [76]. Lp(a) polymorphism is grouped into six main groups depending on the apo(a) isoform bound to the apoB of the Lp(a). The apo(a) isoforms were identified based on their krigle IV repeats andthe number of kringle repeats determines the size of apo(a). The phenotype group were therefore, named as F (with 11-13 kringle IV repeat), B (with 14-16 kringle IV repeat), S1 (with 17-19 kringle IV repeat), S2 (with 20-22 kringle IV repeat), S3 (with 23-25

kringle IV repeat), S4 (>25 kringle IV repeat) [77]. Black people were reported to have higher plasma levels of Lp(a) than white people [72], with a high prevalence of the S4 phenotype, followed by the S3S4 heterozygous phenotype [78]. However, the differences in the apo(a) allele frequencies alone do not explain the known wide variation in the plasma levels of Lp(a)in different ethnic groups [71]. The variation in Lp(a) size may account for approximately 40% of the differences in its serum concentration among Caucasians[79], A study by Marcovina *et al*. (1993), reported that apo(a) polymorphism explained 35% of the variability in Lp[a] concentrations in Caucasians and 27% of the variability in African Americans [80].

The plasma concentrations of Lp(a)vary much more in the African Americans than in Caucasian and Chinese populations. The size polymorphism appears to contribute much less to the inter-individual variability in plasma concentrations of Lp(a) in the African Americans due to the greater variance in plasma Lp(a) levels in this population [81].

There are indications of an inverse relationship between apo(a) size and plasma Lp(a) levels in Caucasian [71] and in black subjects [81]. However, Carstens *et al*. (1998), reported no relationship between the apo(a) size and plasma Lp(a) levels in black subjects [82]. It has become apparent that the apo(a) gene effect is only partial and that other factors other than the isoform size may play a role in regulating the plasma levels of Lp(a) in blacks [82]. The factors responsible for difference in distribution of Lp(a) levels have not been identified, but studies have suggested that the difference in apo(a) isoform size distributions are not solely responsible for difference in distribution of Lp(a) levels [80, 82, 83].

Frohlich (1993) reported that subjects with smallLp(a) isoform sizes(apo (a) with kringle IV repeat of Ò22 are regarded as small apo(a) isoforms and > 22 kingle IV repeats are regarded as large)are more at risk of developing CVD and as a result he suggested that the measurement of apo(a) size may be a useful predictor of CVD especially in individuals without other major risk factors for CVD [79]. When Lp(a) is present in high levels in the plasma, it is recognized as an independent risk factor for premature atherosclerotic cardiovascular disease by mechanisms that are not yet well defined [71].

There are several suggested ways in which high Lp(a) levels can result in CVD. One of the mechanisms is mediated through the endothelial surface layers [84]. Apo(a) bears close structural similarities to plasminogen, thus a high plasma Lp(a) level can interfere with known plasminogen functions in the fibrinolytic system leading to a decreased generation of plasmin, suggesting a thrombogenic action of Lp(a) [78]. Lp(a) competes for the binding of plasminogen to its membrane receptor in endothelial cells and platelets, thus resulting in impairment of the generation of plasmin by fibrin-bound tissue-type plasminogen activator [85]. Also Lp(a) can induce the production of tissue plasminogen activator inhibitor by endothelial cells, an action that can promote attenuation of clot lysis which results in plaque formation leading to cardiovascular events [85].

Another mechanism is mediated through the sub-endothelial intima layers [86]. Lipoprotein (a) can transverse the vascular endothelium and in hyper-lipoprotein(a)proteinaemia, the plasma gradient would favour the transfer and accumulation of Lp(a) in the arterial intima. As a function of its residence time, it is likely that Lp(a) undergoes chemical changes either due to formation of complexes with components of the intima (proteoglycans and fibrin) or oxidative events via oxygen-free radicals [86]. These modified Lp(a) particles would become atherogenic by preferentially entering the macrophages that reside in the intima and by promoting their transformation to foam cells, potential precursors of the atherosclerotic plaque thus leading to CVD [86, 87].

A difference in serum concentrations of lipids and lipoproteins between African Americans and Caucasians, even at an early age was reported in the USA. African Americans exhibited lower triacylglycerol and higher HDL-C concentrations than Caucasians; hence African Americans exhibited a favorable lipid profile compared to Caucasians [88]. In obese Bolivian children and adolescents it was reported that obese children and adolescents had an adverse lipid profile than non-obese children and adolescents (55.7% had low HDL prevalence and 42.6% had increased triglycerides) [89], while a study among 10-19 year old diabetic youngsters in USA reported a23% prevalence for high triglyceride levels and 16% for low HDL-C levels [90]. An earlier study also in USA among adolescents aged 12-19 years reported a 23%prevalence of triglyceride levels and a 23% prevalence of low HDL-C levels [91], similar prevalences of increased

triglycerides and low HDL levels were reported in both studies despite one study being done in diabetic youngsters and the other in non-diabetic youngsters. The studiesô results may suggest that adverse lipid profile is becoming apparent in youngsters and this could to a large extent be attributable to the high prevalence of overweight by both studies.

2.2 Inflammation

Inflammation is a basic way in which the body reacts to infections, irritation or other injury and is characterized by features such as redness, warmth, swelling and pain. Inflammation is now recognized as a type of non-specific immune response [92]. Evidence show that inflammation plays an important role in the pathogenesis of atherosclerosis and that hyperlipidaemia can initiate and enhance the inflammatory response [20, 93].

The development of CVD through inflammation results as a response to haemodynamic stress and other types of injury. In such an inflammatory environment the LDL particle modifying cellular processes become activated, resulting in the modification of LDL particles through oxidation. The products released in the form of free radicals will damaged more LDL particles and may worsen the inflammation [94]. The modified LDL particles will then be engulfed by macrophages which may cause foam cell formation resulting in atherosclerosis which can lead to cardiovascular events such as angina, heart attacks and stroke [94].

Systemic inflammation was first interpreted as an inflammatory response to the developing atheromatous vascular damage. However, an alternative explanation could be that systemic inflammation causes atherosclerosis rather than being the result of it. This interpretation is supported by the observation that patients with pre-existing inflammatory disease have a dramatically increased risk of cardiovascular disease at a younger age [20]. Ridker *et al*. (1997), reported that base-line plasma C-reactive protein concentrations were higher among men who went on to have myocardial infarction or ischemic stroke [95]. Elevated plasma concentrations of C-reactive protein have been reported in patients with acute ischemia [96]

Cardiovascular disease is associated with an increase in markers of low grade inflammation, including C-reactive protein (CRP) and components of the coagulation cascade [20]. Hypertrophic adipocytes and stromal cells within adipose tissue directly augment systemic inflammation and mediate multiple pathogenic mechanisms in the well-known but poorly understood association between obesity, cardiovascular pathology and comorbidities such as dyslipidaemia, type 2 diabetes mellitus, hypertension and metabolic syndrome [20].

Recent evidence shows that some aspect of innate immunity is preserved in adipose tissue and that adipocytes act like macrophages, activate multiple inflammatory signal transduction cascades, and induce and secrete a number of potent inflammatory cytokines and acute phase reactants [20, 97]. An elevated CRP level is associated with obesity and is a risk factor for cardiovascular diseases, increased blood pressure and type 2 diabetes[20]. Larkin *et al*. (2005), reported that there is no significant ethnic difference in CRP levels in adolescents but that CRP varies significantly with BMI [98].

In adolescents CRP levels were increased in the presence of several metabolic abnormalities and in overweight children with the metabolic syndrome, CRP concentrations were higher than in overweight children without the metabolic syndrome [99]. A study in Germany also reported significantly higher serum levels of CRP in obese children compared to non-obese children of the same age, sex and pubertal status and that weight loss is associated with a significant decrease of CRP [100]. Valesquez-Meyer *et al*. (2008), reported that subjects with higher grade of inflammation exhibited higher BMI [101].

Cytokines are small, nonstructural proteins used for regulation of host response to infections, immune responses, inflammation and trauma. There are two classes of cytokines; we have the pro-inflammatory cytokines (promote inflammation) and anti-inflammatory cytokines (suppress the activity of pro-inflammatory cytokines) [102]. Pro-inflammatory cytokines (interleukins and TNF alpha) increases the synthesis of platelet- activity factor leukotrienes; facilitate the passage of leukocytes from the circulation into the tissues; they are inducers of endothelial adhesion molecules [102]. The activation of pro-inflammatory cytokines promotes inflammation and results in increased adhesion by leukocytes to the arterial walls and increased influx of

macrophages to the affected site(s), thus increasing the possibilities of foam cell formation which result in atheromatous plaques that characterize the early stages of atherosclerosis and increase the risk of CVD [103].

2.3 Insulin resistance

Insulin is the central regulator of glucose and lipid homoeostasis [104]. Insulin decreases blood glucose concentrations by reducing hepatic gluconeogenesis and glycogenolysis and by enhancing glucose uptake into muscles and adipocytes [104]. Insulin enhances triglyceride synthesis in the liver and adipose tissue, increases the breakdown of circulating lipoproteins by stimulating lipoprotein lipase activity in adipose tissue and suppresses lipolysis both in adipose tissue and muscles [104]. Insulin resistance can be defined as a condition where physiologic concentrations of insulin are unable to properly regulate the above mentioned processes in glucose and lipid homoeostasis [105].

There are two methods used in the diagnosis of insulin resistance (IR).

One of these methods is the Euglycemic Insulin Clamp method which is said to be the best or gold standard method. Insulin is infused at a particular rate, while glucose is infused at a rate necessary to maintain euglycemia [106]. Under steady-state condition of euglycemia, the rate of exogenous glucose infusion is equal to the rate of insulin-stimulated glucose disposal [106]. The insulin sensitivity or resistance is determined from the amount of glucose required to maintain euglycemia [106].

In the second method the Homeostasis model assessment-Insulin resistance (HOMA-IR) formula is used to estimate insulin resistance. This is calculated as: (fasting serum insulin (μU /ml) × fasting plasma glucose (mmol/l))/22.5 [107]. The HOMA-IR value of above 2.5 is defined as an insulin-resistant state and values less than 2.5 as an insulin-sensitive state in healthy subjects [107]. The HOMA-IR values provides reasonable estimates of insulin resistance in all fasting

samples[107], the current study used the HOMA-IR formula to estimate insulin resistance based on the physiological assumptions and easy practicality of the use of the HOMA-IR formula.
Insulin resistance and hyperinsulinaemia are associated with elevated blood concentrations of plasminogen activator-1, with limitation of fibrinolysis or induction of a prothrombotic state, failure of endogenous fibrolysis and thus increased risk of cardiovascular events [108]. Also because plasminogen activators are potent proteolytics and are important in facilitating vascular smooth muscle migration, relative inhibition of this proteolytic system may inhibit degradation of extracellular matrix and migration of vascular smooth muscle cells [108]. This may render the atheromatous plaques relatively hypocellular, with a high lipid to vascular smooth muscle cell content [108].

Fasting serum insulin concentration exceeding 20 μU/l is indicative of hyperinsulinaemia [58]. Hyperinsulinaemia represents the pathophysiologic step between insulin resistance and impaired glucose tolerance, however data obtained in adult men and children indicate that fasting hyperinsulinaemia is independently associated with various parameters relevant to cardiovascular risk assessment [58, 109, 110].

Hyperinsulinaemia has not only been associated with adiposity that already exists, but with the rate of weight gain in children aged 5-9 [58]. The prevalence of hyperinsulinaemia is higher among children with obesity [12]. Higher total plasma cholesterol and triglyceride levels as well as lower contributions of high-density lipoprotein cholesterol to total plasma cholesterol, were found in hyperinsulinaemic compared to non-hyperinsulinaemic children [58]

A study by Valesquez-Meyer *et al*. (2008), reported a 35% of impaired glucose metabolism, where the indexes of beta cell activity (Insulinogenic index and the composite insulin sensitivity index) and insulin action were equally affected and the prevalence of impaired glucose metabolism was similar in both African Americans and Caucasians [101]. Lee *et al*. (2006), reported no differences in adjusted HOMA-IR between black and white children, however, obese children despite their ethnicity had significantly higher levels of insulin resistance compared with children of normal weight in adjusted comparisons (mean HOMA-IR 4.93[95% Confidence Interval 4.56-5.35 vs. 2.30 [2.21-2.39]. respectively) [34]. Pima Indians were found to be 17%

more insulin resistant than Caucasians after accounting for any differences in degree of obesity. During oral glucose tolerance testing, mean plasma insulin concentrations were 33% higher in the Pima Indians, but these differences were largely explained by the greater insulin resistance in the Pima Indians [111]

2.4 Diabetes mellitus

2.4.1 Type 2 diabetes mellitus

Type 2 diabetes mellitus is a disease that was considered to occur at the onset of adulthood or maturity, but is now presenting in children [104]. The emergence of type 2 diabetes in youth, in recent years has paralleled a rising prevalence of obesity [72] and of insulin resistance and its associated risk factors during childhood and adolescence [103].

Type 2 diabetes mellitus is diagnosed using either the World Health Organization (WHO) or American Diabetes Association (ADA) criteria.

The WHO criterion uses the oral glucose tolerance test (OGTT) method. When conducting the OGTT, subjects are required to fast for at least 8 hours. The subject will be asked to drink a solution containing 75grams of glucose. Blood samples will be taken before the intake of the 75grams glucose solution and again every 30 to 60 minutes after taking the solution. The test may last up to three hours [112].

The OGTT is used to determine a subjectôs glucose tolerance status, thus determining whether a subject has type 2 diabetes mellitus or impaired glucose tolerance (IGT).A 2h glucose tolerance test is usually performed and a glucose level of above 11.1 mmol/l after the 2h test is regarded as diabetic [113], while a glucose level between 7.8 mmol/l and 11.1 mmol/l is regarded as IGT [113], a glucose level less than 7.8 mmol/l is regarded as normal glucose tolerance [113].

The ADA criterion uses the fasting plasma glucose method. A fasting plasma glucose level of Ó 7.0 mmol/l is regarded as diabetic [105], while IFG (impaired fasting glucose) is defined as fasting glucose between 5.6 mmol/l and 6.9 mmol/l [114].

Hyperglycaemia in diabetes mellitus is associated with the generation of reactive oxygen species that play an important role in the pathogenesis of diabetic cardiovascular complications such as myocardial infarction, stroke and peripheral vascular disease [115, 116]. Many biochemical pathways affected by hyperglycaemia can increase the production of free radicals resulting in oxidative stress [108]. Hyperglycaemia and oxidative stress activate the nuclear transcription factor kappa B resulting in the appearance of cell surface adhesion molecules, increased adherence of monocytes and the production of monocyte chemo-attractant protein-1 and other mechanisms that activate chronic low-grade inflammation known to augment atherosclerosis [108].

Protein glycation especially in LDL and Lp(a) together with oxidative stress can alter and damage protein structure [117].These modified protein particles would become atherogenic through their accelerated deposition and macrophages recruitment, facilitating sub-endothelial foam cell formation which will automatically result in plaque formation and possibly cardiovascular events [117].

Diabetic patients have been shown to have a high frequency of atherosclerosis, leading to increased risk of stroke and/ or myocardial infarction [118]. Increased free radical activity has been reported in patients with type 2 diabetes mellitus [118]. Tissue damage by free radicals (oxidative stress) is thought to be an important factor in the pathogenesis of diabetes mellitus and its complications [118].

Individuals with diabetes mellitus are 2-4 times more likely to die of heart disease than those without diabetes mellitus, and cardiovascular disease is responsible for 70% of diabetes mellitus related deaths [119]. Lipoprotein abnormalities have been identified among the several risk factors that could account for the increase in CVD in diabetes mellitus patients [120]. It has been found that reduced levels of apoA-1 are associated with an increase in coronary events [120].

Studies have shown that type 2 diabetes mellitus predominantly affects young females than young males, and that young females were more insulin resistant than young males during puberty and adolescence [104, 121]. A study in Brazil reported that adolescents with obesity or two or more risk factors for type 2 diabetes mellitus had higher insulin resistance and increased ϐ-cell function than those who were non-obese or with zero or one risk factor [122].

The prevalence of childhood type 2 diabetes has increased in the past 15 years [121], and may account for 8% to 45% of new cases of diabetes in youth [123]. Among African American youth aged 10ï 19 years, prevalence (per 1,000) of type 2 diabetes was 1.06 (0.93ï 1.22) and annual incidence (per 100,000) was 19.0 (16.9ï 21.3) [124]. Among 15- to 19-year-old North American Indians, prevalence of type 2 diabetes per 1000 was 50.9 for Pima Indians, 4.5 for all US American Indians, and 2.3 for Canadian Cree and Ojibway Indians in Manitoba, Among African Americans and whites aged 10 to 19 years in Ohio, type 2 diabetes accounted for 33% of all cases of diabetes [121].

2.4.2 Maturity Onset Diabetes of the Young (MODY)

Maturity Onset Diabetes of the Young (MODY) is a clinically heterogeneous group of disorders characterized by non-ketotic diabetes mellitus, an autosomal dominant mode of inheritance, an onset usually before the age of 25 years and mostly in childhood or adolescence and the primary defect in the function of the beta cells of the pancreas leading to beta cells dysfunctions and diabetes mellitus. The beta cells dysfunctions result from different gene mutations that are expressed in the beta cells and the mutations results in different forms of the maturity onset diabetes of the young (MODY1, MODY2, MODY3, MODY4, MODY5) [125].

The following gene mutations have been shown to cause the condition.

- Gene coding for transcription factors hepatocytes nuclear factor 4 alpha (MODY1) [126]
- Gene coding for the enzyme glucokinase (MODY2) [127]
- Gene coding for hepatocyte nuclear factor 1 alpha (MODY3) [128]
- Gene coding for insulin promoter factor 1 (MODY4) [129]

- Gene coding for hepatocyte nuclear factor 1 beta (MODY5) [130]

2.5 Hypertension

Hypertension is defined as a blood pressure exceeding the 95th percentile for age and gender in adolescents [131] and a blood pressure greater and equal to 140/90 in adults [132].
Hypertension is a major contributor to CVD [133]. Once established, hypertension carries a greater risk for development of CVD in obese compared to non-obese individuals [134].

Hypertension is associated with altered vascular structure due to increase arterial pressure [135]. Increased arterial stiffness is a key and a potentially lethal trigger in the etiology of CVD [136]. Arterial stiffening initiates a series of adverse cardiovascular events that include additional increases in central and peripheral arterial systolic blood pressure and pulse pressure and these changes cause or contribute to vascular endothelial injury, which initiates inflammation and the formation of a plaque resulting in increased risk of vascular occlusive diseases or CVD [137].

Obesity is associated with hypertension, and android obesity in particular is an independent risk factor for the development of hypertension [22]. The amount of visceral fat correlated positively with systolic blood pressure levels in centrally obese women [138]. Elevated lipolytic rate of abdominal fat increases the free fatty acid (FFA) blood levels, leading to insulin resistance and hyperinsulinaemia which then enhance adrenergic activity and renal sodium reabsortion, which might be responsible for increases in arterial blood pressure in centrally obese patients [138].

A study carried out in Africa observed a higher prevalence rate of hypertension in the black urban population than in the rural population and this was blamed on high salt intake and low green vegetable consumption [139]. Vegetables contain a significant amount of magnesium, calcium and potassium, which are important in the prevention of hypertension [139]. Hall *et al*. (2000), postulated that sodium retention is not caused by hyperinsulinaemia but would result from disturbances in the renal medulla consequent to a compressive effect determined by the

accumulation of visceral fat thus leading to an activation of the renin-angiotensin system and consequent increased sodium re-absorption, which leads to hypertension [140].

Danish children and adolescents have been shown to have higher levels of blood pressure compared to other nationalities [5]. In Turkey the prevalence of arterial blood pressure exceeding the 95th percentile was 4.4% in 14-18 year old adolescents [141], while the prevalence of arterial blood pressure exceeding the 95th percentile was 6.0% in age group of 14-19 years in USA [142]. Studies have shown positive association between obesity and hypertension [22, 35, 138]. A study conducted in USA showed a high and increased prevalence of overweight and obesity in youngsters and adolescents [34]. Obesity could be playing an important role in the prevalence of hypertension amongst these study groups.

In the Bogalusa Heart Study it was reported that the influence of age on blood pressure is greater in blacks than in whites for children, adolescents and adults and the rate of change in blood pressure from childhood indicates that the mechanisms that predispose blacks to hypertension are functioning in childhood [143]. It was further reported that obesity is an important determinant of higher blood pressure levels and that this association begin in childhood but its influence on blood pressure was more pronounced in whites [143].

2.6 Obesity

Healthy weight, overweight and obesity are defined according to the magnitude of BMI, expressed as weight in kilograms divided by the square of height in meters (kg/m^2) [144]. The measurement of BMI is a practical and reproducible method for classifying overweight in adults [145]. BMI has been defined into classes by international criteria like the World Health Organization (WHO), refer to table 2.6.1 [146] and National Institute of Health (NIH), refer to table 2.6.2 [147]:

Table 2.6.1: BMI definition by WHO.

BMI (kg/m^2)	Classification

<18.5	Underweight
18.5- 24.9	Normal weight
25.0- 29.9	Overweight
>/= 30.0	Obese

Table 2.6.2: BMI definition by NIH

BMI (kg/m^2)	Classification
<19.0	Underweight
19.0ÓX<25.0	Normal weight
25.0 ÒX> 30.0	Overweight
Ó30.0	Obese

The measurement of body fat is difficult both in clinical applications and population studies done in children and adolescents. Thus in general weight adjusted for height is used rather than a more direct measure of body fat [148]. The BMI of children and adolescence is compared with the BMI of a reference population of children of the same sex and age [149]. The 2000 CDC growth charts is widely used, these charts can be found on the internet (www.cdc.gov/growthcharts) and in CDC`s Epi Info software program, where both exact percentiles and z-scores can be calculated [149]. These CDC charts/reference values were recommended for use by the WHO [146]. Table 2.6.3 describes the definition of BMI in adolescents.

Table 2.6.3: BMI definition by CDC growth charts

BMI for age and gender	Classification
< 5^{th} percentile	underweight
85^{th} percentile> X Ò5^{th} percentile	Normal weight
85^{th} percentile ÒX < 95^{th} percentile	Overweight
Ó95^{th} percentile	Obesity

Although BMI correlates well with measures of adiposity and is associated with both mortality and some chronic diseases [150, 151, 152], it does not account for the wide variation in body fat distribution that exists at any level of relative body size [150]. However, waist circumference

compensates for this limitation of BMI by bringing regional fat into consideration [153]. After controlling for BMI it was reported that increased intra-abdominal adipose tissue was strongly associated with metabolic and cardiovascular risk in a variety of chronic diseases, such as stroke, type 2 diabetes mellitus [154] and hypertension [138]. According to international criteria, central obesity in adults is measured as waist hip ratio of greater than 0.90 (men) and 0.85 (women)by the WHO [146], waist circumference of greater than 94cm (men) and 80cm (women) by the IDF [155] and waist circumference of greater than 102 cm (men) and 88cm (Women) by the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III [156].

Obesity contributes to CVD via all other risk factors for CVD such as dyslipidaemia, inflammation, hypertension, insulin resistance and type 2 diabetes mellitus [20]. Mortality rate due to diabetes, hypertension, and acute myocardial infarction are increased dramatically in parallel with the prevalence of obesity in adults and children [157].

Growing evidence shows that it is body fat distribution, and not the absolute amount of adipose tissue, which leads to the increased risk of cardiovascular disease observed in many overweight individuals [20, 158, 159], suggesting that the intra-abdominal visceral fat plays a major role in the process. Visceral fats have been shown to secrete far greater amount of the two inflammatory markers, IL-6 and TNF-Ů, compared to dissected subcutaneous adipose tissues [20, 160] and subjects with an increased amount of abdominal fat have increased levels of cytokines and number of platelets [20]. The activation of cytokines promotes inflammation and results in increased activation and adhesion to vessel walls by platelets thus increasing the possibilities of foam cell formation which result in atheromatous plaque that characterize the early stages of atherosclerosis and increasing the risk of CVD [160, 161].

2.6.1 Obesity and risk factors in young age groups

The quantity and distribution of body fat and blood pressure and blood lipids are all affected by puberty and the changes are influenced by the decrease in physical activity and changes in eating habits that are commonly seen during adolescence [157]. Puberty is a dynamic period of development marked by rapid changes in body size, shape and composition [162]. Body

compositional changes, including the regional distribution of body fat are large during the pubertal transition [162], and factors influencing this pubertal transition are nutritional, genetic and hormonal factors [162] and physical activity [163]. It was reported that changes in eating habits in adolescents towards high fat diet have a negative influence in their body fat and lipid profiles [164].

Thus puberty is a crucial time for the development of risk factors for CVD [157]. The association between adiposity and CVD risk factors is corroborated further by the observation that the prevalence of multiple cardiovascular risk factors is substantially higher in children with obesity than in age-matched non-obese control individuals [165].

A study undertaken in Mexico reported that the frequency of high blood pressure, overweight, obesity, smoking and physical inactivity was higher in adolescent from urban areas while the frequency of dyslipidaemia, mainly low levels of high-density lipoprotein and high levels of triglycerides were higher in rural areas and the risk factors were related to BMI, triceps skin fold and waist circumference [166]. Furthermore, it was reported that overweight was common and ascribed to dietary changes, including increased consumption of high fat, high carbohydrate energy-dense foods, decreased consumption of fruits and vegetables, adoption of a western diet and low physical activity [167]. Increases in fat intake and purchases of sugar and refined carbohydrates, particularly soda soft drinks, may be related to increases in overweight, obesity and mortality due to diet-related causes [168].South African studies reported a decrease in total carbohydrate, increases in animal protein and total fat intake observed in urban population [169] and in rural populations [170].

2.7 Stunting

Malnutrition has been reported as one of the major causes of stunting in children. When food intake is inadequate, energy is conserved by first limiting social activity and cognitive development, then by limiting the energy available for growth [171]. The consequences of stunting in early life, includes increased susceptibility to infectious diseases [172], reduced

height and increased risk of child and adult obesity [37].Studies have reported that stunted children are more at risk of developing CVD risk factors later in life, risk factors may include but not limited to obesity, hypertension, dyslipidemia and diabetes [37, 173]. Change in diet has been reported to predispose stunted children to increase prevalence of CVD risk factors later in life [174]. Mamabolo *et al*. (2006), reported increased stunting in children residing in Capricon District Limpopo province [175]. Growth stunting is defined as height for age below the fifth percentile on a reference growth chart, refer to table 2.7.1 [176].

Table 2.7.1: Stunting category in percentile

Gender	Cut off	category
Male	< 5^{th} percentile	stunted
Female	< 5^{th} percentile	stunted

2.8 Lifestyle

2.8.1 Alcohol consumption and cigarette smoking

The prevalence of smoking [177] and alcohol consumption is rapidly increasing among the younger generation [178]. Association between alcohol consumption and CVD mortality varied with the amount consumed: light to moderate drinking was related to lower CVD mortality, while heavy drinking was associated with increased CVD mortality [178].

Evidence indicates that flavanoids in alcohol may influence many factors that participate in the formation and evolution of atherosclerosis plaques [178]. Flavanoids are known to be powerful antioxidants, that counteracts the oxidation of LDL which plays a significant role in the development and progression of atherosclerotic plaques. However, the benefits of flavanoids comes with the moderate use of alcohol while excessive use of alcohol has an adverse effect in the lipid profile resulting in dyslipidaemia (hypertriglyceridaemia and increased LDL-C) which can mimic plaque formation [178].

Cigarette smokers had a significantly increased risk of CVD-specific mortality relative to non-smokers [179]. Cigarette smoking was observed not only to offset the protective effect of moderate drinking but also to strengthen the harmful effect of heavy drinking [179]. Fatty streaks and fibrous plaque in the aorta and coronary arteries are however already detectable in children and young adults who smoke [179]. Cessation of smoking results in a reduction in fibrinogen and white blood cell count and an increase in HDL-C [180].

Cigarette smoking decreases the level of high-density lipoprotein cholesterol and increases the level of low-density lipoprotein cholesterol [180]. Cigarette smoking does not only result in an atherogenic lipid profile but also generates oxidized LDL with its greater propensity for atheroma formation [181]. These oxidized LDL particles potentially contribute to atheroma progression by promoting monocyte adhesion to the endothelium, decreasing nitric oxide mediated endothelial vasodilation, activating macrophage phagocytosis and foam cell formation resulting in plaque formation as such leading to cardiovascular events [21, 182].

2.8.2 Physical inactivity

Physical inactivity appears to increase the risk of developing coronary artery disease, and much evidence suggests that regular exercise even to a moderate degree reduces this risk and decreases mortality. Exercise can also help modify other risk factors for atherosclerosis by lowering blood pressure and cholesterol levels and by promoting weight loss and decreasing insulin resistance [36].

Physical inactivity is associated with an increase in cardiovascular events in both men and women. Physical inactivity is highly prevalent in urban subjects and is established as an important risk factor for cardiovascular disease [36]. Being physical active is associated with about 40 to 50% reduction in cardiovascular disease. This reduction appears to be statistically independent of blood pressure and other known cardiovascular risk factors [36].

Even though there is a high prevalence of obesity among women residing in Dikgale DSS, a high number of steps/day was reported in rural women, a mean of 9085±4014 steps per day was

recorded in the study population across all ages, however, a mean of 10273± 4385 steps per day was recorded for women under the age of 24 years [183].

3 METHODOLOGY

3.1 Study population and methodology

3.1.1 Study design

The study was across-sectional study.

3.1.2 Study area

The study took place in the Dikgale Demographic Surveillance Site (DDSS), situated 20km from the University of Limpopo (Turfloop campus) and 45-50km north-east of Polokwane, the capital city of Limpopo Province. The site consists of 8(eight) villages which are rural settlements where the dwellings are a mix of shacks, conventional brick houses and traditional mud huts. These villages are typical of those found in rural areas. They are characterized by a very high level of unemployment and such reflects a transition between traditional and modern lifestyles since many younger adults work in neighboring towns and cities and return home for periodic visits. Each village is surrounded by land, where cattle graze and where the community farms maize and vegetables. They mostly rely on rain water for irrigation. The villages have poor sanitation and water supply. A Primary Health Care (PHC) clinic is situated in the DDSS and the regional hospital is approximately 22 km from the PHC clinic. The villages have dusty roads and a poor transportation system [184].

3.1.3 Study Population

The present study is part of the ñGene-Environment Interaction Projectò in which 830 participants aged 15 years and above participated. Participants (n=830) were recruited from households in the Dikgale DDSS between December 2005 and December 2007. Before commencing the survey, local community chiefs were visited to explain the purpose of the study, request permission to recruit participants from the communities and to have leaders inform the communities about the survey. A random sample of 1000 subjects was generated from the DDSS relational database and distributed to the trained field workers. Initially the field workers

reported difficulty in contacting the participants during house- to- house visits. Therefore, in view of time and financial constraints it was decided that field workers would also recruit participants at common meeting places and through general word-of-mouth promotion of the survey. Signed consent was obtained from all participants. The current sub-study was approved by the Hospital Complex Ethics Committee in 2008 (Limpopo Department of Health and Social Development).

After participants had consented to the study, fieldworkers administered questionnaires to each participant. Questionnaires administered by fieldworkers were aimed at obtaining information about tobacco and alcohol use. Thereafter, the fieldworkers made arrangements with the participants regarding the date and the venue for blood collection.

For the present study participants between the ages of 15-20 years were sampled from the ñGene Environment Interaction Projectò database. Of the 830 participants who participated 250 were between 15-20 years of age. Of the 250participants, 23 participants did not fast and were excluded. Of the remaining 227 participants, 12 did not have glucose values and 10 did not have insulin values and 2 did not have triglyceride values and this left 203 participants with complete data. Of the 203 participants 7 had CRP levels of above 10 mg/l and 10 had creatinine levels above 130 mmol/l as such they were excluded. Thus the total population size for this study was 188 participants with 101 males and 87 females.

3.1.4 Exclusion criteria

Participant under the age of 15 years and above age 20 years were excluded. Participants with CRP levels of Ó 10 mg/l(this was meant to exclude participant with infectious diseases) and creatinine levels >130 mmol/l were excluded (this was meant to exclude participants with kidney failure).

3.1.5 Data Collection

Participants were asked to fast overnight before the morning of blood collection. Blood was collected between 6H30 to 8H30 or longer, depending on the number of participants who came that morning.

i. Blood collection

Experienced, registered, qualified nurses were employed to collect fasting blood samples from participants, using the following tubes:

(a) EDTA-containing tubes for plasma.
(b) Sodium fluoride tubes for glucose determination stored in a cooler with ice until lab analysis.
(c) Tubes without anticoagulant for serum.

Immediately after the blood samples were collected they were placed in a cooler box with ice ready to be taken to the laboratory, the samples were taken within two hours to the laboratory to be centrifuged.

ii. Anthropometric measurements

Anthropometric measurements (weight, height, waist and hip circumference) were taken by trained fieldworkers.

<u>Waist, hip circumferences and waist hip ratio</u>

A non-stretchable tape was used to measure the waist and hip circumferences. The waist was measured at the level of the umbilicus while the hip circumference was measured at the level of the groin fossa. Participants were in a relaxed state and wore light clothing when the measurements were taken. From these measurements, the waist-hip ratio (WHR) was calculated as waist over hip circumference.

Table 3.1: Cut off points for waist and WHR by gender

	Waist	WHR
Females	<88 cm	<0.85

Males	< 102 cm	<0.90

Weight, height and BMI

For height and weight measurements participants wore light clothing and were barefooted.

Height

Each participant was attended by one trained personnel. The height was measured to the nearest 1.0cm using a stadio-meter. This was placed against the wall and participants were asked to stand with their backs against it. Participants were requested to remove their shoes and hats. The back of the head, shoulder blades, buttocks, and heels make contact with the backboard of the stadio-meter. Height measurements were made with the head aligned in the Frankfort horizontal plane. The stadio-meter headpiece was lowered that it rests firmly on top of the participantôs head, with sufficient pressure to compress the hair and measurements were recorded.

Weight

The weight was measured to the nearest 1.0 kg, using a digital balance. The digital balance was calibrated against Mechanical Physician Scale (Detecto 439) manufactured by Detecto Corporation. Participants were asked to slowly mount the balance and stand upright and reading recorded.

BMI

The weight and height measured were used to calculate the body mass index.
Formula: BMI= weight / $(height)^2$
Participants 18 years and above the definition by WHO to define weight was used:

Table 3.2: WHO BMI definition for adults

BMI (kg/m^2)	Classification
<18.5	Underweight
18.5- 24.9	Normal weight

25.0- 29.9	Overweight
>/= 30.0	Obese

Participant below 18 years definition of weight, percentile classification by CDC growth charts (in program EPI-INFO) were used:

Table 3.3: WHO BMI definition for adolescents

BMI for age and gender	Classification
< 5th percentile	underweight
85th percentile > X Ò5th percentile	Normal weight
85th percentile ÒX < 95th percentile	Overweight
Ó95th percentile	Obesity

iii. Blood pressure

Blood pressure was measured by using Omron M5-I (Omron Corporation).Participants were allowed to sit on a chair for five minutes after which three measurements were taken and the mean of the last two readings was used. Hypertension was defined as blood pressure Ó 140 (SBP) and Ó90 (DBP) for adolescent from age 18 years and above. Hypertension was defined as a blood pressure exceeding the 95th percentile for age and genders in adolescent below 18 years of age (determined in EPI-INFO program) and a blood pressure greater and equal to 140/90 in adolescents above 18 years of age [131].

iv. Physical activity

A pedometer (NL-2000, New Lifestyles Inc., Kansas City,MO,USA) was used to determine the average number of steps per day taken by each participant. The pedometer was worn on the right side of the waist, securely attached to a nylon belt and sealed with a surgical tape. The pedometers could be removed for sleeping and bathing purposes. The pedometers were worn for nine consecutive days, so that when pedometers were collected they contained step totals from

seven full days, which could be recalled for each day and recorded. Average number of steps/day were calculated for this seven day period.

The pedometers were calibrated and validated before being handed to participants. Participants were asked whether they wore the units properly (as instructed) during waking hours of the 7 days. If participants replied negatively or could not provide accurate times or days for non-compliance, their data was excluded from the analysis. The following criterion was used to determine physical activity: sedentary lifestyle (<5000 steps/day), low-somewhat active (5000-9999 steps/day) and active to very active (Ó10000 steps/day) [185].

v. Blood analysis

In the laboratory,the blood sampleswere immediately centrifuged (Beckman Coulter, Allegra X-22R, 1204 x g-force for 20 min) and the plasma and serum obtained were stored at -70°C until analyzed. Glucose measurements were made immediately after separation.

The following equipments were used for biochemical tests:

1. ILAB 300-plus Chemistry System (Instrumentation Laboratories, Italy) for the measurement of triglyceride, total cholesterol, creatinine, HDL-C and glucose.
2. Immage NephelometrySystem (Beckman Coulter,USA) for the measurement of hsCRP, ApoB, ApoA, Lp(a).
3. Access Immunoassay chemistry System (Beckman Coulter, USA) for the measurement of insulin.
4. LDL-C was calculated using the Friedwald formula (LDL= TC-HDL-TG/2.17 (mmol/l)).

Routine laboratory practices were followed to maintain the equipments. The calibrators and controls used were in good working order and when expired were discarded. For every run, controls were included.

It must be noted that although most of the data was obtained from the existing database, stored serum was used to perform additional biochemical tests (ApoA-1, ApoB, CRP, T-cholesterol) that were needed for the present study.

3.2 Statistical analysis

Data was analyzed using SPSS 14 for Windows. Individuals were categorized according to gender and descriptive statistics were computed and expressed as mean (SD), while variables that were not normally distributed were expressed as median and interquartile ranges. Data with abnormal distribution was log transformed to improve normality of the distribution. Regression analysis was carried out to determine the association between anthropometry, age and gender with lipids, blood pressure and Insulin resistance.

Differences in mean values between groups (male vs. females) were assessed by the t-test. The Chi-square test was used to compare differences in the prevalence of CVD risk factors by gender. Statistical significance was set at a probability of $p<0.05$.

The 2000 CDC growth charts for adolescents are widely used, these charts can be found on the internet (www.cdc.gov/growthcharts) and in CDC`s Epi Info software program, where both exact percentiles can be calculated [141]. These CDC charts/reference values were recommended for use by the WHO [138]. For the current study Epi-info program version 3.4.3 was used for adolescents under 18years of age as recommend by WHO.

4 RESULTS

Results of anthropometric and blood pressure measurements stratified by gender are presented in Table 4.1. All normally distributed variables are represented as means ± SD and not normally distributed variables are presented as the median (interquartile range).

Table 4.1 Descriptive analysis of anthropometric and blood pressure measurements of the participants by gender.

Variables (Ref ranges)	**Reference ranges**	**Total population (N=188)**	**Males (N=101)**	**Females (N=87)**	**P-value**
Age (yrs)		17.19± 1.78	17.13 ± 1.76	17.25 ± 1.80	0.66
Height (cm)		162.24 ± 10.12	165.66 ± 9.89	158.27 ± 8.90	<0.0001
Body mass (Kg)		55.11 ± 9.48	53.68 ± 8.79	56.77 ± 10.02	0.03
BMI (Kg/m^2)	<25.0	21.03 ± 3.94	19.51 ± 2.43	22.80 ± 4.60	<0.0001
Waist (cm)	male: < 102 cm; Female: < 88 cm	70.12 ± 8.20	67.66 ± 6.75	72.97 ± 8.82	<0.0001
Hip (cm)		89.25 ± 9.67	85.31 ± 7.46	93.82 ± 9.95	<0.0001
WHR	Male < 0.90; Female < 0.85	0.79 ± 0.08	0.79 ± 0.05	0.78 ± 0.11	0.37
Table 4.1. (Cont.)					

Variables (Ref ranges)	**Reference ranges**	**Total population (N=188)**	**Males (N=101)**	**Females (N=87)**	**P-value**
Average steps/day	>5000 step/day	14129 ± 5872	15428 ± 6003	12564 ± 5338	0.01
SBP	< 95th percentile <140 mm/Hg	109.39 ± 12.98	111.56 ± 13.59	106.87 ± 11.83	0.01
DBP	< 95th percentile <90 mm/Hg	70.26 ± 8.70	69.67 ± 9.30	70.94 ± 7.90	0.32

The mean values for all the variables were within the normal ranges (Table 4.1.) After stratifying the study population according to gender, females had significantly higher mean values for body mass (p=0.03), BMI (P<0.0001), waist (P<0.0001), hip-circumference (P<0.0001) compared to males, while males had a significantly higher mean values for height (P<0.0001), average step/day (P<0.0001), and SBP (P=0.01), compared to females.

Results of biochemical measurements stratified by gender are presented in Table 4.2. All normally distributed variables are represented as means ± SD and not normally distributed variables are presented as the median (interquartile range).In the table 4.2, trig, Lp(a), insulin, HOMA-IR and hs CRP were presented as median (interquartile ranges).

Table 4.2: Descriptive analysis of biochemical parameters of the participants by gender.

Variables	**Reference ranges**	**Total population (N=188)**	**Males (N=101)**	**Females (N=87)**	**P-value**
T-chol	<5.00 mmol/l	3.62 ± 0.73	3.56 ± 0.67	3.70 ± 0.78	0.21

Table 4.2. (cont.)

Variables	**Reference ranges**	**Total population (N=188)**	**Males (N=101)**	**Females (N=87)**	**P-value**
HDL-chol	Males> 1.0mmol/l; females>1.2 mmol/l	1.24 ± 0.33	1.31 ± 0.33	1.18 ± 0.31	0.006
LDL-chol	< 3.0 mmol/l	1.96 ± 0.58	1.86 ± 0.52	2. 90 ± 0.62	0.005
Trig	<1.7 mmol/l	0.77(0.58;1.14)	0.77(0.59;1.10)	0.76(0.58;1.17)	0.36
ApoB	males: 63-133mg/dl; females: 60-126mg/dl	62.18 ± 21.84	59.20 ± 19.46	65.55 ± 24.01	0.05
ApoA-1	males: 94-178mg/dl; females: 101-199 mg/dl	105.28 ± 28.50	109.22 ± 28.85	100.34 ± 26.87	0.03
ApoB/apo A-1	males: 0.4-1.25; females: 0.38-1.07	0.63 ± 0.26	0.57 ± 0.23	0.70 ± 0.29	0.001
TC/HDL-C	<5.0	3.04 ± 0.79	2.83 ± 0.63	3.29 ± 0.88	<0.0001

*Lp(a)	<30mg/dl	28.20(13.42;61.88)	28.80(13.10;54.85)	27.80(14.30;70.90)	0.97
Glucose	<5.6 mmol/l	4.57 ± 0.56	4.64 ± 0.64	4.47 ± 0.47	0.04
*Insulin	<20µU /ml	6.63(4.25;13.51)	6.23(3.98;16.16)	7.62(4.58;12.10)	0.95

Table 4.2. (Cont.)

Variables	**Reference ranges**	**Total population (N=188)**	**Males (N=101)**	**Females (N=87)**	**P-value**
°Beta cell function		136.03 (86.18;235.86)	123.4 (73.69;274,43)	152.82 (94.52;250.24)	0.55
*HOMA-IR	< 2.5	1.34(0.84;2.70)	1.26(0.75;2.88)	1.53(0.87;2.37)	0.74
*hs-CRP	<3.0mg/l	0.58(0.20;1.73)	0.32(0.20;1.08)	0.85(0.36;2.18)	0.001

*variables that used median and interquartile ranges.° nine subject excluded due to low Glucose number of less than 3.5, therefore 179 subject were used to determine beta cell function (male=97 and females=82).

The mean values for all the variables were within the normal ranges (Table 4.2.) After stratifying the study population according to gender. Females showed a significantly higher mean values for LDL-C (P=0.005), apoB/apoA-1 ratio (P=0.001), and T-chol/HDL-C (P<0.0001), hs-CRP value (P=0.001) compared to males, while males showed significantly higher HDL-C (P=0.006), glucose (P= 0.04) and apoA-1(P=0.001) levels.

The prevalence of risk factors for CVD among the total population and by gender is presented in Table 4.3.For adolescents below the age of 18, valuesbetween85thand 95thpercentile were used as cutoff values to determine the prevalence of overweight and values at and above 95th percentile were used as cut off values to determine the prevalence of hypertension, obesity and central obesity, while, adolescent 18 and above the normal adult ranges and cutoff points were used.

Table 4.3 Prevalence of risk factors in the total population and by gender.

Risk factors	**Reference ranges**	**Total population (n=188) % (n)**	**Females (n=87) % (n)**	**Males (n=101) % (n)**	**P-value**
Overweight	BMIÓ85th and <95th percentile or BMI > 25kg/m^2 and Ò30 kg/m^2	6.4 (12)	12.6 (11)	1.0 (1)	<0.0001
Obesity	BMIÓ95th percentile or BMI> 30kg/m^2	4.3 (8)	9.2 (8)	(0)	<0.0001
Central obesity	Waist Ó 95th percentile or female>88cm Male> 102	0	0	0	--
*Sedentary lifestyle	Av step<5000	3.3 (6)	4.8 (4)	2.0 (2)	0.14
Stunting		26.5 (45)	16.7 (13)	34.8 (32)	0.009
Insulin resistance	HOMA>2.50	29.3 (55)	23.0 (20)	34.7 (35)	0.11
Hyperinsulinaemia	Insulin>20 µU/l	13.3 (25)	8.0 (7)	17.8 (18)	0.55
IFG	Glucose, 5.6-6.99 mmol/l	4.3 (8)	1.1 (1)	6.9 (7)	0.09

Diabetes	Glucose Ó7.00 mmol/l	0.5 (1)	0 (0)	1.0 (1)	0.09
Table 4.3 (cont.)					
Risk factors	**Reference ranges**	**Total population (n=188) % (n)**	**Females (n=87) % (n)**	**Males (n=101) % (n)**	**P-value**
Hypertension	BPÓ95th percentile or BPÓ140/90	8.5(16)	5.7(5)	10.9(11)	0.16
High apoB/apoA-I ratio	>0.9	16.5 (31)	26.4 (23)	7.9 (8)	0.001
High TC/HDL-C	>5.00	2.1 (4)	3.4 (3)	1.0 (1)	0.34
High T-Chol levels	>5.00 mmol/l	4.0 (8)	6.5 (6)	1.9 (2)	0.25
High LDL-C levels	>3.0 mmol/l	4.8 (9)	8.0 (7)	2.0 (2)	0.08
High Trig levels	>1.7 mmol/l	5.3 (10)	6.9 (6)	4.0 (4)	0.52
Low HDL-C levels	Ò 1.0 mmol/l Male and Ò 1.2 mmol/l Female	34.6 (65)	55.2 (48)	16.8 (17)	<0.0001
High hs-CRP	HsCRP>3.00 mg/l	15.4 (29)	18.4 (16)	12.9 (13)	0.32

*Smoking		10.8 (20)	2.4 (2)	17.8 (18)	0.001

Table 4.3 (Cont.)

Risk factors	**Reference ranges**	**Total population (n=188) % (n)**	**Females (n=87) % (n)**	**Males (n=101) % (n)**	**P-value**
**Alcohol use		11.4 (21)	2.4 (2)	18.8 (19)	<0.0001
Metabolic Syndrome (IDF criteria)[130]		0	0	0	-
Metabolic Syndrome (ATP III criteria)[129]		1.1 (2)	2.3 (2)	0 (0)	0.21

*= a total of 183 participants participated (84=F, 99=M), **= a total of 185 participants participated (84=F, 101=M). Criteria for metabolic syndrome according to IDF and ATP are found in Tables 2.1 and 2.2 respectively (pages 3-4).

In the total population the range in the prevalence of anthropometry determined risk factor was: stunting with 26.5%, overweight with 6.4% and obesity at 4.3% of the study population. Females showed a significantly high prevalence of overweight (12.6% vs. 1.0%, $p<0.001$) and obesity (4.3% vs. 0.00%, $p<0.001$) compared to males, while, males showed a significantly higher prevalence of stunting (34.8% vs. 16.7%, $p=0.009$) compared to females. Low physical activity was observed in 3.3% of the study population, with no significant difference between groups ($p=0.14$).

In the total population the range in prevalence of risk factors was: decreased HDL-C levels with 34.6%, insulin resistance with 29.3%, increased apoB/apoA-1 ratio with 16.5%, increased hs-

CRP levels with 15.4% and hyperinsulinaemia with 13.3% of the study population. This was followed by a 10.8% and 11.4% prevalence of smoking and alcohol use respectively. No participants with metabolic syndrome were observed in this population when using the IDF criterion. Only 1.1% of the total population had metabolic syndrome when using the ATP III criterion, with no significant difference between groups (p=0.21). Females showed a significantly higher prevalence of low HDL-C levels (55.2% vs. 16.8%, P<0.001), high apoB/apoA-I ratio (26.4% vs. 7.9%, p=0.001). Males showed a significantly higher prevalence of stunting (34.8% vs.16.7% p=0.009), tobacco (17.8% vs. 2.4%, p=0.001) and alcohol use (18.8% vs. 2.4%, p=0.001) compared to females. Males had a higher but not statistically significant prevalence of HOMA (34.7% vs. 23.0%, p=0.11), hyperinsulinaenia (8.0% vs. 17.8%, p=0.55) and IFG (1.1% vs. 6.9%, p=0.09) compared to females.

Regression analysis was done to determine if any association exists between the traditional CVD risk factors and gender, age, anthropometric variables and physical activity. Tables 4.4, 4.5 and 4.6 show the association between anthropometry and serum lipids, blood pressure and insulin resistance.

Table 4.4: Association between lipids and age, gender, anthropometry and Insulin.

Dependent Variable	Independent variable	Beta-coefficient	P-value
TC/HDL-C	Gender	-0.22	0.006
	Age	0.14	0.04
	BMI	0.14	0.13
	Waist	0.04	0.63
	Physical activity	-0.08	0.28
	Insulin	0.09	0.20
	Glucose	0.05	0.45
HDL-C	Gender	0.17	0.04
	Age	-0.22	0.003
	BMI	-0.05	0.59
	Waist	0.02	0.83
	Physical activity	0.05	0.47
	Insulin	-0.09	0.21
	Glucose	0.01	0.88

Table 4.4. (Cont.)

Dependent Variable	Independent variable	Beta-coefficient	P-value
TC	Gender	-0.04	0.62
	Age	-0.07	0.34
	BMI	0.12	0.20
	Waist	0.08	0.35
	Physical activity	0.02	0.22
	Insulin	-0.03	0.68
	Glucose	0.07	0.36
Trig	Gender	-0.05	0.55
	Age	-0.11	0.16
	BMI	0.04	0.72
	Waist	-0.02	0.81
	Physical activity	-0.06	0.43

	Insulin	0.11	0.17
	Glucose	-0.01	0.88
LDL-C	Gender	-0.13	0.11
	Age	0.07	0.33
	BMI	0.16	0.07
	Waist	0.10	0.24
	Physical activity	0.01	0.86
	Insulin	-0.03	0.73
	Glucose	0.08	0.25
ApoB/apoA-1	Gender	-0.15	0.06
	Age	-0.13	0.08
	BMI	0.09	0.28
	Waist	-0.05	0.54
	Physical activity	-0.25	0.001
	Insulin	-0.02	0.77
	Glucose	-0.06	0.38

Table 4.4 (Cont.)

Dependent Variable	**Independent variable**	**Beta-coefficient**	**P-value**
ApoB	Gender	-0.04	0.57
	Age	-0.18	0.01
	BMI	0.15	0.10
	Waist	-0.04	0.61
	Physical activity	-0.25	0.001
	Insulin	-0.04	0.62
	Glucose	-0.04	0.60
ApoA-1	Gender	0.17	0.05
	Age	-0.04	0.64
	BMI	0.08	0.38
	Waist	0.02	0.83

	Physical activity	0.11	0.16
	Insulin	0.02	0.78
	Glucose	0.04	0.62

P-valve<0.05 is regarded as significant

Gender and age showed an association with the lipid variable HDL-C. Physical activity showed a negative significant association with ApoB/A-1 and apoB.

Table 4.5: Association between blood pressure and age, gender and anthropometry.

Dependent Variable	**Independent variable**	**Beta-coefficient**	**P-value**
SBP	Gender	0.21	0.008
	Age	0.13	0.08
	BMI	0.09	0.33
	Waist	0.12	0.15
	Physical activity	0.21	0.005

Table 4.5 (cont.)

Dependent Variable	**Independent variable**	**Beta-coefficient**	**P-value**
DBP	Gender	-0.04	0.64
	Age	-0.02	0.76
	BMI	0.11	0.23
	Waist	-0.03	0.77
	Physical activity	-0.02	0.88

P-value<0.05 is regarded as significant

Gender and physical activity showed significantly positive association with systolic blood pressure. No independent variables showed an association with diastolic blood pressure.

Table 4.6: Association between insulin resistance, Insulin and glucose and age, gender and anthropometry.

HOMA IR	Gender	0.18	0.02
	Age	0.09	0.21
	BMI	-0.002	0.98
	Waist	0.17	0.05
	Physical activity	0.01	0.88
Insulin	Gender	0.17	0.04
	Age	0.10	0.17
	BMI	-0.002	0.97
	Waist	0.19	0.04
	Physical activity	0.02	0.80
Glucose	Gender	0.15	0.07
	Age	-0.07	0.36
	BMI	0,07	0.47
	Waist	0.004	0.96
	Physical activity	0.005	0.94

P-valve<0.05 is regarded as significant

Gender showed a significantly positive association with insulin resistance. Gender and waist showed a significant positive association with Insulin.

5 DISCUSSION

Studies have shown an increase in the prevalence of CVD risk factors in adult populations both in developed and developing countries [15, 186], which may in the near future lead to an increased prevalence of CVD events and stroke in adults. It has been shown that CVD risk factors are not only present in adult populations but are also becoming prevalent in young people [5, 6, 7].

The current study population has been shown to be undergoing health transition [15, 175]. Determining the prevalence of CVD risk factors in adolescents especially in rural populations is important since it may alert the health authorities of the need to introduce measures to prevent future increases in the prevalence of CVD events and stroke in South African rural populations.

a. Obesity

Obesity in children and adolescents has been related to development of other CVD risk factors such as hypertension [35], dyslipidaemia [30], insulin resistance [34], type 2 diabetes mellitus [33] and inflammation [31, 32] later in life. South African studies have shown a high prevalence of obesity in adult populations both in urban and rural areas [15, 186]. The South African Demographic and Health Survey (2003), reported a high overall prevalence of overweight and obesity in SA especially in women, with more than 24% of adolescent women and 9% of adolescent men being classified as either overweight or obese. The prevalence was higher in urban populations compared to rural populations [186].

Similarly, Alberts *et al*. (2005), conducted a study among an adult rural black population and reported similar results, where 29% of men and 59% of women were either overweight or obese [15]. Obesity is not only limited to the adult population but appears to start at a younger age [186]. International studies have shown a high and increasing prevalence of overweight and obesity in youngsters and adolescents [34, 187].

The present study showed a low prevalence of overweight (6.4%) and obesity (4.3%) when compared to international studies [30, 31, 34] and a comparable prevalence when compared to a local study [188]. Studies reported an overall low fat diet in South Africa and lowest fat intake were found in participants living in rural areas and highest fat intake were found in participants living in urban areas [189,190]. The difference in obesity and overweight between local studies and international studies could be due to the low physical activity and high fat diet observed in international studies.

However, the prevalence of overweight (12.6%), and obesity (9.2%) in females was significantly higher than in males (overweight (1.0 %) and obesity (0%)). The results in females are similar to the reported findings by Puoane *et al*. (2005), who reported that 10% of South African women were obese at the age of 15-24 years [191]. Mukuddem *et al*. (2004), reported that even though they found no significant association between stunting and overweight in 10-15 year old children, there was a tendency for girls older than 15 years to start to gain subcutaneous fat, even though at this age they were still stunted [192]. Studies showed that obesity appears to start in women at a young age, possibly due to female hormonal changes which are reported to promote fat deposition [193, 194] in the lower extremities, especially in black women and result in larger proportion of body mass as fat [195].

Madhavan *et al*. (2007), reported that adolescent boys in a rural area of South Africa are more physically active since herding and other tasks as well as their own excursions away from the village take them away from the home where there is food available, and thus boys are more frequently fending for themselves than girls [196]. Whether the same applies to the present study population is not known. The present study data also show males to be more physically active than females as indicated by the significantly higher ($p<0.01$) number of steps by males than females. The regression analysis showed that obesity had no association with HDL-C or with apoB/apoA-1 ratio in the present population.

Boure *et al*. (2002), reported that the proportion of fat intake has increased while that of carbohydrate has decreased in both rural and urban areas [172]. However, a previous study has shown low fat intake in the study area [190].

b. Hypertension

Hypertension has become a common cause of heart failure and stroke in Africans [197]. Studies in South Africa have reported a high prevalence of hypertension in the adult black population both in rural and urban areas [15, 198]. This is not limited only to the adult population but also occurs at a younger age [197]. A study done in rural South African children aged 6-13 years reported a similar prevalence of hypertension to the present study. Hypertension was observed in 4.8% of boys and 5.1% in girls. The prevalence of hypertension was reported to be evident in girls, while that of overweight was low [199].

The present study showed a slightly lower prevalence of hypertension (8.5%) compared to data reported in an international study (9.4%)[200] and a local study [201]. The local study done in North West province of Republic of South Africa reported a 21.3% and 33.1% prevalence of prehypertension/hypertension in girls and boys, respectively [201]. The study further reported no significant association between blood pressure and BMI and lifestyle factors, however, peripheral fat was strongly and positively related to SBP in girls while centrally located fat showed a stronger association with SBP [201]. Current study also showed no significant association between BMI and waist circumference, but Systolic blood pressure was shown to be significantly associated with gender and physical activity. However, it has been shown that there is an increase in hypertension parallel to the increase in obesity in youngsters [200].

The prevalence of hypertension in the present study was comparable between gender groups despite females showing high prevalence of overweight and obesity. A review of the South African data from 1995-2005, suggested that the noxious effect of obesity in black people is less than in people from other population groups [202]. Although the prevalence of obesity was higher among females in the present study, both genders showed a similar prevalence of hypertension. Regression analysis showed that obesity had no association with blood pressure in this study population.

Ethnicity has been reported to be a determinant of hypertension and black participants were reported to have a higher prevalence of hypertension compared to other ethnic groups [203, 204].

The birth to ten study carried out in South Africa, showed that black children had a higher prevalence of increased blood pressure compared to Indian and white children [205]. However, the mechanism and causal factors of this phenomenon have not yet been fully established.

A study by Cruickshank *et al*. (2005), reported an increase in hypertension in black adolescents compared to white adolescents and it was further reported that the ethnic differences in blood pressure that emerged so powerfully between black and white Americans during adolescence can be accounted for by differences in intrauterine growth, as indicated by birth weight followed by slightly lesser effects of early weight gain [206].

According to the Barker hypotheses [207], slow fetal growth could program or permanently alter the body`s structure and physiology in ways linked to CVD in adults. Barker proposed that influences in the fetal environment could also affect blood pressure in adult life [207]. A review by Alexander (2006), suggested that the reduction in nephron number during nephrogenesis may lead to a decrease in glomerular filtration rate (GRF) and a subsequent reduction in pressure natriuresis resulting in hypertension [208].

Adverse conditions *in utero* may lead to low birth weight (LBW) and fetal programming of hypertension and CVD. During fetal life, tissues and organs go through critical periods of development, thus when under-nutrition occurs *in utero* at a critical or sensitive period of development. The resulting adaptive changes may be permanent and lead to long-term changes in structure and function [208]. Limpopo province is known to be one of the poorest provinces in South Africa, with a high prevalence of LBW which is one of the top five causes of infant death [209]. Mothers living in deprived socioeconomic conditions were reported to frequently have low birth weight infants [210].

Furthermore, Svetley *et al*. (1996), suggested that blacks may have an abnormal transport mechanism of sodium and low renin activity. A study in USA showed blacks to have increased salt sensitivity [211], which results in low excretion of salt in the body. High intake of sodium in South Africa particularly in poor settings have been reported, as it is used to preserve food and to make food tastier [202]. A positive association between salt intake and hypertension has been

reported [212], hence, a high intake of sodium may be contributing to the high prevalence of hypertension in this rural area.

Therefore, under-nutrition which results in LBW, ethnicity and high salt intake may be the contributing factors to the development of hypertension shown in the present study.

c. Insulin resistance (IR), Hyperinsulinaemia and Diabetes Mellitus

Insulin resistance is known to be the initial step in the development of type 2 diabetes mellitus [213] and eventually CVD. Lee *et al*. (2006), reported IR to be positively associated with obesity in adolescents [34]. In the present study population there was a high prevalence of insulin resistance but no association was observed between IR and BMI, these findings supports those by Olefsky *et al*. (1981), who reported that adiposity does not explain the relative insulin resistance in Africans [214]. Beta cell function was normal in the majority of participants in the current study population with few subjects having values below 100% functionality.

The high prevalence of IR is consistent with other studies among black subjects [34, 215]. However, there are no clear mechanisms to explain why black subjects presents with an increased prevalence of insulin resistance as also evaluated by Lovejoy *et al*. (1992), who reported increased insulin resistance in black women compared to Caucasians despite their low visceral fat [216]. This is also congruent with South African results where it was reported that black obese women demonstrated a higher degree of insulin resistance despite less visceral fat compared to white obese women [217]. Black children [218] and adults [219] had lower insulin response than white children and white adults respectively. The physiological cause for greater IR among blacks has not yet been identified [219].

A high prevalence of malnutrition in South African especially in rural populations has been found. It was further reported that malnutrition during pregnancy could lead to low birth-weight babies, who are not only at increased risk of mental and physical underdevelopment but also programmed to be at increased risk of CVD later in life [220]. High blood pressure and glucose levels without adult obesity and even complete catch-up growth in subjects who have been

reported to have had low-birth weight has been shown. It was further reported that a significant number of subject reported to have had low birth weight exhibited a phenotype of glucose intolerance and increased blood pressure together with insulin resistance and beta-cell hyper-function as compared to subjects with normal birth weight [221]. Similar finding were also observed by Hofman *et al*. (1996), who reported high insulin resistance in low birth weight pre-pubertal children as compared to their normal birth weight peers [222].

Studies have shown deficiencies in essential nutrients required during pregnancy in women of childbearing age residing in Limpopo Province [223]. The risk for deficiencies is further increased during pregnancy as women enter pregnancy with inadequate nutrient stores and are thus unable to withstand the demands imposed by the growing fetus [224]. Failure to meet these nutrient requirements could result in negative outcomes including martenal mortality or/and low birth weight [225]. Mmamabolo *et al*. (2007), showed that 34% of children were stunted at 1 year. They further showed significantly lower birth weights and ponderal indices in stunted compared to the non-stunted children [226]. Furthermore, another local study done in Limpopo Province reported a 8.8% of infant low-birth weight, 9.6% of the infants were stunted at birth, 48.9% were underweight and 7.3% were wasted [227]

The increase in IR in the present study may also be explained by Barker`s hypothesis: During fetal life, tissues and organs go through critical periods of development, thus under-nutrition might have occurred *in utero* in these adolescents. Since this is a critical or sensitive period of development, the resulting adaptive changes may have been permanent and led to this early observation of IR, which in the long run may result in hypertension and insulin resistance and type 2 diabetes mellitus later in life [207].

Regression analysis showed that obesity had no association with insulin resistance in the present study. Even though there is no exact explanation for the increased IR in blacks, fetal environment and ethnicity may be playing important roles. The high prevalence of IR may predispose the study population to becoming diabetic in the future, since IR is thought to represent an initial step in the pathogenesis of type 2 diabetes mellitus [34].

The present study showed a 13.3% prevalence of hyperinsulinaemia with a comparable prevalences between gender groups. Weiss *et al*. (2006), reported that insulin clearance was lower in African Americans than in subjects of white origin, thus contributing to higher peripheral insulin concentrations [228]. Thus blacks were reported to have lower levels of C-peptide to insulin ratio than white adolescents [229]. The increased insulin levels may be the result of the low insulin clearance and the pancreatic ϐ-cells compensation for IR by increasing insulin secretion leading to hyperinsulinaemia which is a way to maintain homeostasis [34, 230].

Type 2 diabetes mellitus is an increasing chronic disease among the adult black population in South Africa both in urban and rural areas, with a high prevalence in women [15]. In Pima Indian adolescents aged 15 to 19 years a high prevalence of type 2 diabetes mellitus was observed [230], followed by a slightly lower prevalence of type 2 diabetes mellitus in 12-19 year old Americans [230]. A study done in Kuwaiti reported a low prevalence of type 2 diabetes mellitus in youngsters aged 6-18 years old [231]. Similarly, in the present study a very low prevalence of diabetes mellitus was observed.

d. Inflammation

A high prevalence of subclinical inflammation (15.4%) was observed in the present study population. Females had a non-significant higher prevalence of inflammation (hsCRP) compared to males (p=0.32). The prevalence was low compared to other studies [31, 232, 233]. Overweight children and adolescents were reported to have higher prevalence of inflammation than their lean counterparts, with 26% in obese and 7.9% in non-obese adolescents [31].

Increased physical activity has been reported to decrease CRP levels [233]. The low prevalence of inflammation in the present study population compared to other studies could be due to low prevalence of overweight, obesity and high physical activity in the present study population. Khera *et al*. (2005), reported that blacks have significantly higher CRP levels than white subjects. This difference persisted after exclusion of subjects taking statins and oral estrogen and after adjustment for traditional CV risk factors and BMI [234].

A report by the Medical Research Council of South Africa (2000) reported that Low Birth Weight (LBW) in Limpopo province is one of the top five factors that account for 70% of child deaths [209]. Sattar *et al*. (2004), showed that birth weight is inversely related to CRP concentrations later in life after adjusting for its other known determinants like age, BMI, smoking, socio-ecomonic deprivation and hormone use in women [235]. Though birth weight was not available in the current study, it is still possible that LBW resulting from fetal under-nutrition may have resulted in the increased prevalence of high CRP levels in the present study population. The 15.4% prevalence of high CRP in the current study population may be attributable to fetal under-development (LBW) and ethnicity.

e. Serum lipids

The present study showed low mean values and low prevalence of abnormal serum lipids compared to studies carried on American [30, 203], Iranian [236], Korean [237] and Chinese adolescents [237], except for HDL-C which was comparable. Surprisingly, the present study found a high prevalence of low HDL-C (34.6%) levels and high prevalence (16.5%) of the apoB/apoA-1 ratio of > 0.9.The mechanisms behind the increased prevalence of low HDL-C in this population is unknown and may need further investigation. The increased prevalence of the apoB/apoA-1 ratio may have resulted from the high prevalence of low HDL-C based on the strong positive correlation reported between HDL-C and apoA-1 [64].

A study carried out in Turkey reported low HDL-C values for Turkish children of low socio-economic status [238]. Low HDL cholesterol levels were also observed in the Aboriginal and Torres Strait Islander groups, which was thought to result from the low levels of total cholesterol [239]. The low HDL-C levels in the present study may be due to low cholesterol levels, therefore, HDL-C alone may not be regarded as a good indicator of increased CVD risk in the present study as most subjects had a low total cholesterol. Tchol/HDL-C ratio seems to better explain the risk better. The Tchol/HDL-C ratio was determined in the present data with a cutoff point of greater than 5 [240] and a low prevalence of Tchol/HDL-C (2.1%) was observed. The apoB/A-1 ratio was also determined and found to be 16.5% and significantly higher in females (26.4%) compared to males (7.9%). Female adolescents showed amore unfavorable lipid profile

compared to males, which may render females susceptible to development of CVDs than males in future.

Differences in the lipid levels between obese Teheran adolescents and controls were observed, where obese adolescents presented with significantly higher percentages of abnormal lipid profiles. Obese subjects had increased LDL-C, TC, and triglycerides but had comparable HDL-C levels to the control group [241]. Despite the favorable plasma lipids in the current study population, females showed signs of developing unfavorable plasma lipids, with significantly lower HDL-C and a significantly higher apoB/apoA-1 ratio compared to males. Even though females were reported to be significantly heavier than males, after performing regression analysis to determine whether obesity had any association with the lipids, it was found that obesity had no significant association with HDL-C, T-chol/HDL-C and apoB/apoA-1.

Smith *et al*. (1975), documented that one of the long-range potential concerns regarding the use of oral contraceptives is the increase in serum lipids, they further showed that women in the oral contraceptive group had significantly higher triglyceride concentrations compared to non-oral contraceptive group [242]. It was also reported that in 14-17 year old girls there was an increase in total cholesterol resulting from increased beta-lipoprotein cholesterol, a decrease in alpha lipoprotein cholesterol and an increase in triglyceride in adolescent using oral contraceptives especially in black girls [243]. The use of contraceptives in the present study population was not determined, this could have contributed to the increased LDL-C, and apoB-100 levels, and low apoA-1 levels in females.

Chen *et al*. (2006), reported a decrease in serum lipids and lipoproteins after a high fiber, low fat diet and vigorous exercise [244]. A study by Steyn *et al*. (2001), reported a high carbohydrate and low fat intake in the Dikgale population [190]. The overall low prevalence of abnormal lipid profile in the present study could be due to low fat intake, low overall obesity and high physical activity when compared to previous studies [244]. The difference in the lipid profile between gender groups may be attributable to high physical activity in men and/or the use of oral contraceptives in women.

f. Smoking and Alcohol intake

Smoking may induce changes in the serum lipid levels in an atherogenic direction [38], and Craig *et al*. (1989), observed that smokers have a higher risk of developing CVD compared to non-smokers [245].

The present study reported a low prevalence of tobacco and alcohol use which was 10.8% and 11.4% respectively compared to other studies [246, 247]. A study reported that 29% of girls and 65% of boys used alcohol while 25% of the whole population admitted to tobacco use [246], while, Challier *et al*. (2000), reported that 20.5% of the study population were smokers and 40% used alcohol [247].In the present study males were found to use tobacco and alcohol more frequently than females as was also reported in previous studies [246, 248].

A study in adolescents aged 15-19 years in Limpopo province reported a 10.6% overall prevalence of smoking (16.7% for males and 5.8% for females) and a 39.1% prevalence of alcohol intake (47.9% in males and 32.1% in females) [248]. The present study reported a lower prevalence of smoking and alcohol intake compared to previous studies [246, 248] and reported as similar prevalence of cigarette smoking (17.1% in boys and 0% in girls) to the present study population [249], however, the present study showed exposure to smoking and alcohol use in rural adolescents especially in males.

Even though males were observed to be using tobacco more than females they still showed a better lipid profile compared to females. Regression analysis results showed no association between lipid variables and tobacco use. There was no significant association between tobacco use and HDL, T-chol/HDL-C and apoB/apoA-1.

The percentage of subjects using alcohol was low in the present study population compared to other studies [246, 248], however, there is a fairly high exposure of alcohol intake in the current population especially in males, 18.8% of males indicated that they were using alcohol, while only 2 female subjects (2.4%) indicated that they used alcohol. A study by Dubow *et al*. (2008), reported that people exposed to drinking at an early age are likely to become compulsive alcohol

consumers at a later stage [39]. There is, therefore, the possibility that 11.4% of the study population may end up being compulsive drinkers. The study population is to a great extent strongly holding to their culture and tradition which strongly discourage and condemn the use of alcohol and drugs among the youth. This strong holding to their beliefs and culture may have resulted in under reporting of smoking and alcohol use by the study subjects.

g. Limitation of the study

Limitation of the study is that this was not a complete random selection of subjects. The Study had a small population size. However, the study showed the presence of CVD risk factors among a young rural black population.

Conclusion

The results of the present study showed a high prevalence of several CVD risk factors, while the prevalence of other conventional risk factors was relatively low. The high prevalence of low HDL-C may be due to low total cholesterol levels and a better indicator may be the ratio of total cholesterol/HDL-C, which had a low prevalence in both males and females. The observation that overweight and obesity were only present in females may be partially explained by higher physical activity levels in males compared to females. Regression analyses showed no association between obesity and other risk factors for CVD.

The relatively high prevalence of IR, subclinical inflammation and hypertension may be the result of under nutrition during the fetal stage and/or during early infancy. Nutrition is critical *in utero* and in early infancy, since this is a time where there is major organ development. If under nutrition/malnutrition occurs *in utero* or at a critical or sensitive period of development, the resulting adaptive changes may be permanent and lead to long-term changes in structure and function of organs including the pancreas and the renal system.

Therefore, educational programmes should be implemented to educate rural populations with regard to the importance of balanced nutrition, increasing their physical activity, stopping smoking and alcohol consumption as means of avoiding future development of CVD in rural areas.

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APPENDICES

Appendix I: Principles of Biochemical Tests

Principles of the biochemical tests used:

Glucose determination

Glucose

Glucose concentration was determined by means of an enzymatic reaction,using the ILAB 300-plus Chemistry System(glucose oxidase) with kits supplied by the Instrumentation Laboratories, Italy.Glucose reagent contains: Glucose oxidase, Peroxidase, Phenol, 4- Aminoantipyrine, Phosphate buffer and less than 0.1% sodium azide.

Glucose determination principle:

B-D-Glucose + O_2 $\xrightarrow{\text{glucose oxidase}}$ Gluconic acid + H_2O_2

$2H_2O_2$ + Phenol + 4- Aminoantipyrine $\xrightarrow{\text{peroxidase red}}$ quinoneimine + $4H_2O$

The absorbance of the red dye is proportional to the glucose concentration in the sample. The absorbance was measured at 510nm.

Precision (Coefficient of Variance (CV)): 2.2%
Sensitivity: Milli-absorbance change per 1mg/dl = 2.3
Minimum detection limit: 0.0 mmol/l
Interference: None by Bilirubin, lipemia and heamoglobin

Total cholesterol determination

Cholesterol was measured using the ILAB 300-plus Chemistry System with kits supplied by the Instrumentation Laboratories, Italy.Total cholesterol reagent contains: Cholesterol esterase, Cholesterol oxidase, Peroxidase, Phenol, 4-aminoantipyrine, Tris buffer.

Total cholesterol determinationprinciple.

Cholesterol esters + H_2O cholesterol esterase cholesterol + fatty acids

Cholesterol + O_2 cholesterol oxidase cholest-4-en-3-ene + H_2O_2

$2H_2O_2$ + Phenol + 4-aminoantipyrine peroxidase Red quinoneimine + $4H_2O$

The absorbance generated by the red dye is proportional to the cholesterol concentration in the sample. Absorbance measurement is taken at 510nm.

Precision (Coefficient of Variance (CV)): 1.6%
Sensitivity: Milli-absorbance change per 1mg/dl = 2.0
Minimum detection limit: 0.0 mmol/l
Interference: None by Bilirubin, lipemia and heamoglobin

HDL cholesterol determination

HDL Cholesterol was measured using the ILAB 300-plus Chemistry System with kits supplied by the Instrumentation Laboratories, Italy.HDL-R1 contains: Good`s buffer pH 7.0, 4-aminoantipyrine, Peroxidase, Ascorbate oxidase, Anti-human ϐ-lipoprotein antibody and preservatives. HDL-R2 contains: Good`s buffer pH 7.0, Cholesterol esterase, Cholesterol oxidase, N- ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy-4-fluoroaniline.

HDL cholesterol determination principle:

The HDL-cholesterol assay is a homogeneous, direct method for measuring levels of HDL-C without the need for sample pre-treatment.
The Anti-human ϐ-lipoprotein antibody in R1 binds to lipoproteins (LDL, VLDL AND chylomicrons) other than HDL. The antigen-antibody complexes formed block enzyme reaction with all lipoproteins except HDL-C when R2 is added.
Cholesterol esterase and cholesterol oxidase react only with HDL-C.

Hydrogen peroxide produced by enzyme reactions with HDL-C yields a blue coloured complex upon oxidative condensation of the chromogen. The concentration of the blue coloured complex is proportional to the concentration of HDL-C.
The absorbance was taken at 620 nm.

Precision (Coefficient of Variance (CV)): 1.7%
Sensitivity: Milli-absorbance change per 1mg/dl = 144
Minimum detection limit: 0.03 mmol/l
Interference: None by Bilirubin, lipemia and heamoglobin

Triglyceride determination

HDL Cholesterol was measured using the ILAB 300-plus chemistry system with kits supplied by Instrumentation Laboratories, Italy.Triglyceride reagent contains: Lipoprotein lipase, Glycerol kinase, Glycerophosphate oxidase, Peroxidase, 4-chloro-phenol, 4-aminoantipyrine, ATP, Mg++, Good`s buffer.

Triglyceride determination principle

Triglycerides $\xrightarrow{\text{lipoprotein lipase}}$ glycerol + fatty acids

Glycerol +ATP $\xrightarrow{\text{glycerol lipase}}$ glycerol-3-phosphate + fatty acids

Glycerol-3-phosphate+O_2 $\xrightarrow{\text{glycerol phosphate oxidase}}$ Dihydroxyacetone phosphate + H_2O_2

H_2O_2 + 4-chlorophenol + 4-aminoantipyrine$\xrightarrow{\text{peroxidase}}$red quinoneimine + $2H_2O$

The concentration of the red coloured complex is proportional to the concentration of triglyceride.
The absorbance was taken at 510 nm.

Precision (Coefficient of Variance (CV)): 2.3%
Sensitivity: Milli-absorbance change per 1mg/dl = 133
Minimum detection limit: 0.05 mmol/l
Interference: None by Bilirubin, lipemia and heamoglobin

LDL-Cholesterol determination

The Friedewald formula was used to estimate LDL-C(Friedwald formula: LDL= TC-HDL-TG/2.17 (mmol/L)) [250].

Creatinine determination

IL test TM Creatinine was measured using ILAB 300-plus Chemistry System with kits supplied by Instrumentation laboratories, Italy.Creatinine R1 contains: Sodium hydroxide and Creatinine R2 contains: Picric acid

Creatinine determination principle
Creatinine + Picrate $\longrightarrow$ red complex

The increase in absorbance due to the red complex is proportional to the creatinine concentration in the sample. Absorbance was taken at 510nm.
Precision (Coefficient of Variance (CV)): 3.1%

Sensitivity: Milli-absorbance change per 1mg/dl = 0.26
Minimum detection limit: 0.1 mg/dl
Interference: None by Bilirubin, lipemia and heamoglobin

High sensitivity CRP determination

HsCRP was measured using the Immage Immunochemistry System with kits supplied by Beckman Coulter, USA. The reagent cartridge contains: CRP antibody (particle bound goat and mouse anti-CRP antibody), Sodium Azide (<0.1% (w/w)), diluent.

High sensitivity CRP determination principle:

> The IMMAGE CRPH reagent is based on the highly sensitive Near Infrared Particle Immunoassay rate methodology. An anti-CRP antibody-coated particle binds to CRP in the serum sample resulting in the formation of insoluble aggregates causing turbidity.
> The rate of aggregate formation is directly proportional to the concentration of CRP in the sample. The measure of the rate of increase in light scattering from the complex formed in the solution was proportional to the concentration of the CRP in the sample.

Precision (Coefficient of Variance (CV)): 5.0%
Sensitivity: 0.02- 6.0mg/dl
Interference: None by Bilirubin, lipid and heamoglobin

ApoB determination

Apolipoprotein B was measured using the Immage Immunochemistry System with kits supplied by Beckman Coulter USA. The reagent cartridge contains: ApoB antibody (Processed goat sera), Sodium Azide (<0.1% (w/w)).

ApoBdetermination principle:

The IMMAGE ApoB reagent is based on the highly sensitive Near Infrared Particle Immunoassay rate methodology. An anti-ApoB antibody-coated particle binds to ApoB in the serum sample resulting in the formation of insoluble aggregates causing turbidity. The rate of aggregate formation is directly proportional to the concentration of ApoB in the sample. The measure of the rate of increase in light scattering from the complex formed in the solution was proportional to the concentration of the ApoB in the sample.

Precision (Coefficient of Variance (CV)): 4.0%
Sensitivity: 35- 225 mg/dl
Interference: None by Bilirubin, lipid and heamoglobin

ApoA-1determination

Apolipoprotein A-1 was measured using the Immage Immunochemistry System with kits supplied by Beckman Coulter USA. The reagent cartridge contains: ApoA antibody (Processed goat sera), Sodium Azide (<0.1% (w/w)).

ApoAdetermination principle:

The IMMAGE ApoA reagent is based on the highly sensitive Near Infrared Particle Immunoassay rate methodology. An anti-ApoA antibody-coated particle binds to ApoA in the serum sample resulting in the formation of insoluble aggregates causing turbidity. The rate of aggregate formation is directly proportional to the concentration of ApoA in the sample. The measure of the rate of increase in light scattering from the complex formed in the solution was proportional to the concentration of the ApoA in the sample.

Precision (Coefficient of Variance (CV)): 4.0%
Sensitivity: 25- 225 mg/dl
Interference: None by Bilirubin, lipid and heamoglobin

Lp(a) determination

Lipoprotein (a) was measured using the Immage Immunochemistry System with kits supplied by Beckman Coulter USA. The reagent cartridge contains: Lp(a) antibody (polyclonal anti-lipoprotein (a) antibody; rabbit), Sodium Azide (<0.1% (w/w)).

Lp(a) determination principle:

The IMMAGE Lp(a) reagent is based on the highly sensitive Near Infrared Particle Immunoassay rate methodology. An anti-Lp(a) antibody-coated particle binds to Lp(a) in the serum sample resulting in the formation of insoluble aggregates causing turbidity.

The rate of aggregate formation is directly proportional to the concentration of Lp(a) in the sample. The measure of the rate of increase in light scattering from the complex formed in the solution was proportional to the concentration of the Lp(a) in the sample.

Precision (Coefficient of Variance (CV)): 5.0%

Sensitivity: 2.0- 128 mg/dl

Interference: None by Bilirubin, lipid and heamoglobin

Insulin determination

Insulin was measured using the Access Immunoassay chemistry system, using ultra-sensitive insulin reagent from Beckman.The Insulin reagent contains: Insulin antibody, lumi-phosa 530.

Insulin determination principle:

Insulin + antibody Ÿ Insulin antibody complex + lumi-phosa 530

Ÿ Luminescence.

The luminescence of the immuno complex is measured with a luminometer and its intensity is proportional to the insulin concentration in a sample.

It must be noted that although most of my data was obtained from the existing database, I also used stored serum to perform additional biochemical tests that were needed for the present study.

Precision (Coefficient of Variance (CV)): 4.3%

Sensitivity: 0.03 µIU/ml

Interference: by Pro-Insulin is 0.26% and C-peptide is none

Appendix II:Objectively measured physical activity

OBJECTIVELY MEASURED PHYSICAL ACTIVITY

Pedometer number	

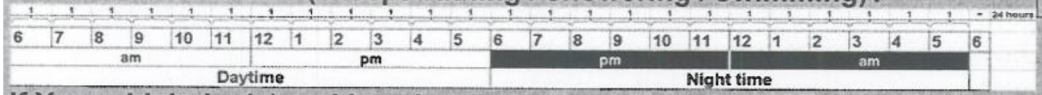
Day of placement (1= M / 2= Tu / 3= W / 4= Th / 5= F / 6= Sa / 7= Su)			
Date of placement (mm/dd/yyyy)	mm	dd	yyyy
Time of placement (hh:mm)	hh	mm	

Day of placement and removal (circle both days)														
M	Tu	W	Th	F	Sa	Su	M	Tu	W	Th	Fr	Sa	Su	M

Day of removal (1= M / 2= Tu /3= W / 4= Th / 5= F / 6= Sa / 7= Su)			
Date of removal (mm/dd/yyyy)	mm	dd	yyyy
Time of removal (hh:mm)	hh	mm	

	Pedometers Readings						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Steps							
Cals							

Except for when the days the monitors were put on and taken off, were there any days or nights during which the pedometer was NOT worn the whole time (except bathing / showering / swimming)?	1 = Yes 2 = No	



If Yes, which day(s) and how long was the pedometer NOT worn (hours)?

Day	Daytime	Night time

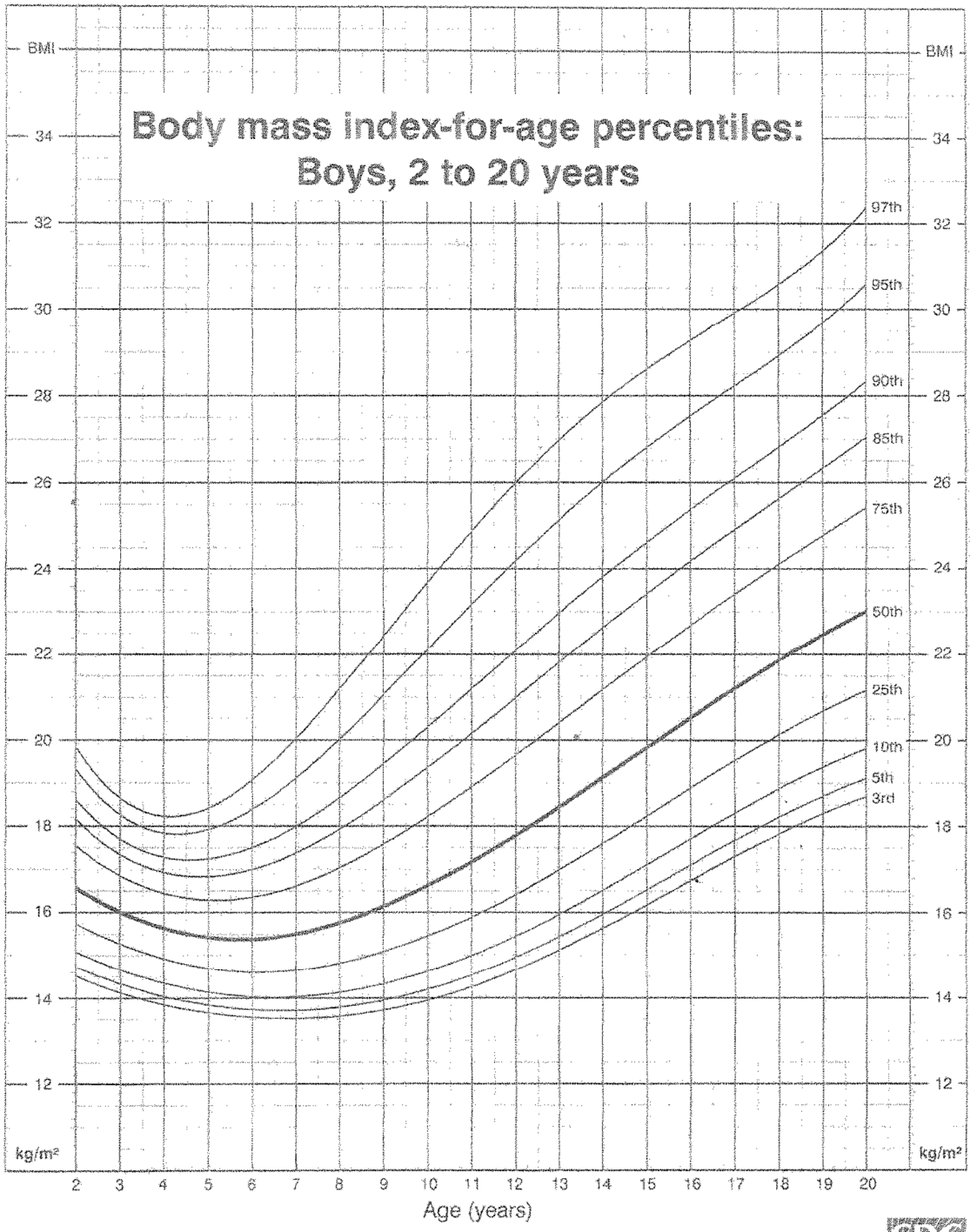
Appendix III: Tobacco Use

CORE Tobacco Use (Section S)					
Now I am going to ask you some questions about various health related habits. This includes things like smoking, drinking alcohol, eating fruits and vegetables and physical activity. Let's start with smoking.					
		Response		Coding Column	
S 1a	Do you currently smoke any **tobacco products**, such as cigarettes, cigars or pipes? Cigarette • Commercial eg "Lucky Strike • Rolled (newspaper + BB/Horseshoe tobacco	Yes No	01 02	☐☐	*If No, go to Expanded Section**
S 1b	<u>If Yes,</u> Do you currently smoke tobacco products **daily**?	Yes No	01 02	☐☐	*If No, go to Expanded Section**
S 2a	How old were you when you **first started** smoking daily?	Age (years) Don't remember	77	☐☐	*If Known, go to S 3*
S 2b	Do you remember how long ago it was?	In Years		Years ☐☐	
		OR in Months		Months ☐☐	
	(CODE 77 FOR DON'T REMEMBER)	OR in Weeks		Weeks ☐☐	
S 3	On average, **how many** of the following do you smoke each day?	Manufactured cigarettes		☐☐	
	(RECORD FOR EACH TYPE)	Hand-rolled cigarettes		☐☐	
		Pipes full of tobacco		☐☐	
	(CODE 88 FOR NOT APPLICABLE)	Cigars, cheroots, cigarillos		☐☐	
	☐☐☐☐☐☐☐☐	← Other (please specify):		☐☐	

Appendix IV: Alcohol Use

CORE Alcohol Consumption (Section A)			
The next questions ask about the consumption of alcohol.			
		Response	**Coding Column**
A 1a	Have you **ever consumed** a drink that contains alcohol such as beer, wine, spirits, *["Black Label, Kudu, Bjala bja Sesotho, Tototo*]?	Yes 01 No 02	☐☐
A 1b	Have you consumed alcohol within the **past 12 months**?	Yes 01 No 02	☐☐
A 2	In the past 12 months, **how frequently** have you had at least one drink? *(READ RESPONSES)* Beer, wine, spirits, *["Black Label, Kudu, Bjala bja Sesotho, Tototo*]?	5 or more days a week 01 1-4 days per week 02 1-3 days a month 03 Less than once a month 04	☐☐
A 3	When you drink alcohol, **on average**, how many drinks do you have during one day?	Number Don't know 7 7	☐☐
A 4	During each of the **past 7 days**, how many standard drinks of any alcoholic drink did you have each day? *(RECORD FOR EACH DAY* *USE SHOWCARD)*	Monday Tuesday Wednesday Thursday Friday Saturday Sunday	☐☐ ☐☐ ☐☐ ☐☐ ☐☐ ☐☐ ☐☐

Appendix V: Male adolescents' body mass index-for-age



Published May 30, 2000.
SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion

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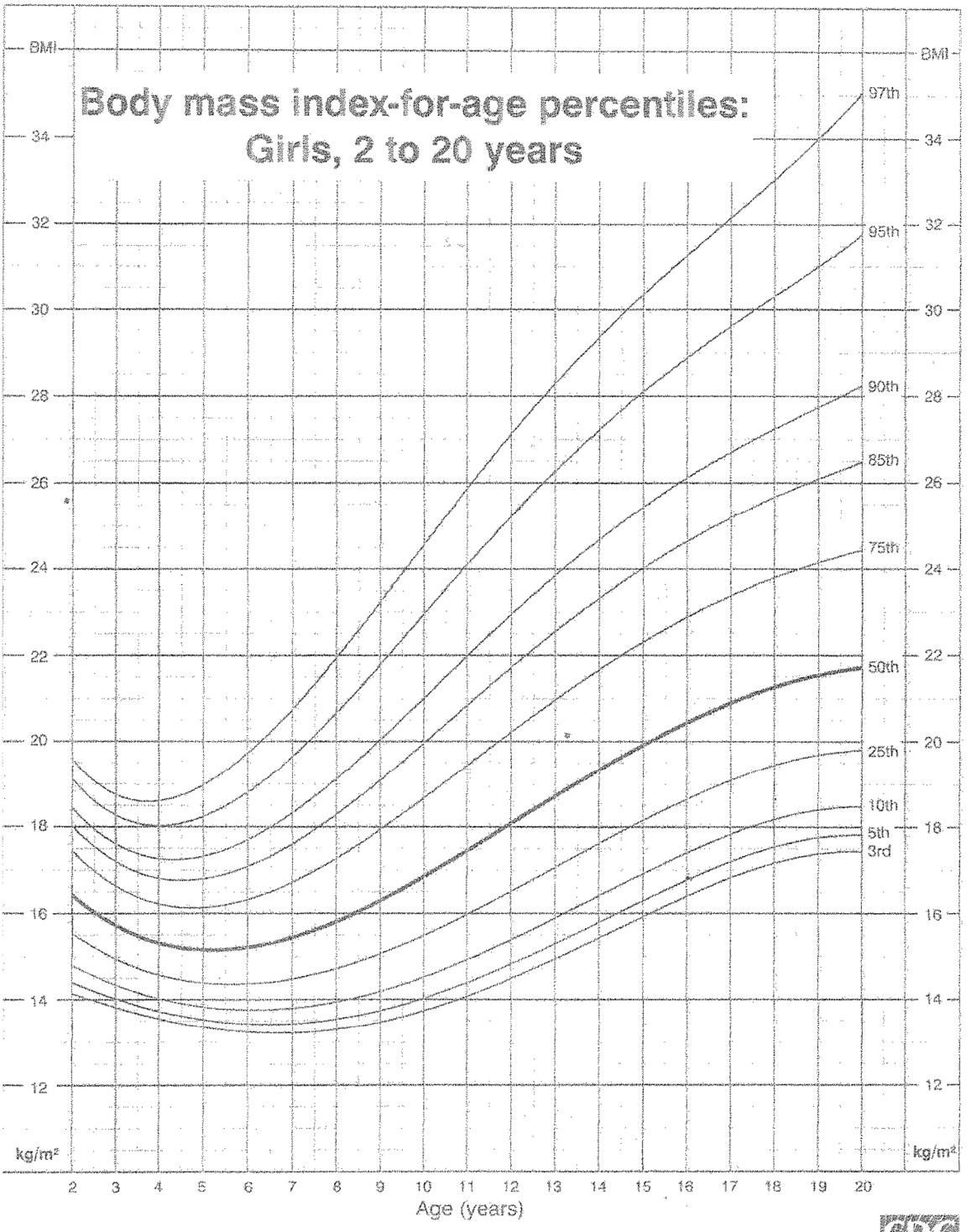
Appendix VI: Female adolescents' body mass index-for-age



Published May 30, 2000.
SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).

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Appendix VII: Male adolescents' blood pressure-for-age

Blood Pressure Levels for Boys by Age and Height Percentile

Age (Year)	BP Percentile ↓	Systolic BP (mmHg)							Diastolic BP (mmHg)						
		← Percentile of Height →							← Percentile of Height →						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	50th	80	81	83	85	87	88	89	34	35	36	37	38	39	39
	90th	94	95	97	99	100	102	103	49	50	51	52	53	53	54
	95th	98	99	101	103	104	106	106	54	54	55	56	57	58	58
	99th	105	106	108	110	112	113	114	61	62	63	64	65	66	66
2	50th	84	85	87	88	90	92	92	39	40	41	42	43	44	44
	90th	97	99	100	102	104	105	106	54	55	56	57	58	58	59
	95th	101	102	104	106	108	109	110	59	59	60	61	62	63	63
	99th	109	110	111	113	115	117	117	66	67	68	69	70	71	71
3	50th	86	87	89	91	93	94	95	44	44	45	46	47	48	48
	90th	100	101	103	105	107	108	109	59	59	60	61	62	63	63
	95th	104	105	107	109	110	112	113	63	63	64	65	66	67	67
	99th	111	112	114	116	118	119	120	71	71	72	73	74	75	75
4	50th	88	89	91	93	95	96	97	47	48	49	50	51	51	52
	90th	102	103	105	107	109	110	111	62	63	64	65	66	66	67
	95th	106	107	109	111	112	114	115	66	67	68	69	70	71	71
	99th	113	114	116	118	120	121	122	74	75	76	77	78	78	79
5	50th	90	91	93	95	96	98	98	50	51	52	53	54	55	55
	90th	104	105	106	108	110	111	112	65	66	67	68	69	69	70
	95th	108	109	110	112	114	115	116	69	70	71	72	73	74	74
	99th	115	116	118	120	121	123	123	77	78	79	80	81	81	82
6	50th	91	92	94	96	98	99	100	53	53	54	55	56	57	57
	90th	105	106	108	110	111	113	113	68	68	69	70	71	72	72
	95th	109	110	112	114	115	117	117	72	72	73	74	75	76	76
	99th	116	117	119	121	123	124	125	80	80	81	82	83	84	84
7	50th	92	94	95	97	99	100	101	55	55	56	57	58	59	59
	90th	106	107	109	111	113	114	115	70	70	71	72	73	74	74
	95th	110	111	113	115	117	118	119	74	74	75	76	77	78	78
	99th	117	118	120	122	124	125	126	82	82	83	84	85	86	86
8	50th	94	95	97	99	100	102	102	56	57	58	59	60	60	61
	90th	107	109	110	112	114	115	116	71	72	72	73	74	75	76
	95th	111	112	114	116	118	119	120	75	76	77	78	79	79	80
	99th	119	120	122	123	125	127	127	83	84	85	86	87	87	88
9	50th	95	96	98	100	102	103	104	57	58	59	60	61	61	62
	90th	109	110	112	114	115	117	118	72	73	74	75	76	76	77
	95th	113	114	116	118	119	121	121	76	77	78	79	80	81	81
	99th	120	121	123	125	127	128	129	84	85	86	87	88	88	89
10	50th	97	98	100	102	103	105	106	58	59	60	61	61	62	63
	90th	111	112	114	115	117	119	119	73	73	74	75	76	77	78
	95th	115	116	117	119	121	122	123	77	78	79	80	81	81	82
	99th	122	123	125	127	128	130	130	85	86	86	88	88	89	90

Appendix VII: Male adolescents' blood pressure-for-age (cont.)

Blood Pressure Levels for Boys by Age and Height Percentile (Continued)

Age (Year)	BP Percentile ↓	Systolic BP (mmHg) ← Percentile of Height →							Diastolic BP (mmHg) ← Percentile of Height →						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
11	50th	99	100	102	104	105	107	107	59	59	60	61	62	63	63
	90th	113	114	115	117	119	120	121	74	74	75	76	77	78	78
	95th	117	118	119	121	123	124	125	78	78	79	80	81	82	82
	99th	124	125	127	129	130	132	132	86	86	87	88	89	90	90
12	50th	101	102	104	106	108	109	110	59	60	61	62	63	63	64
	90th	115	116	118	120	121	123	123	74	75	75	76	77	78	79
	95th	119	120	122	123	125	127	127	78	79	80	81	82	82	83
	99th	126	127	129	131	133	134	135	86	87	88	89	90	90	91
13	50th	104	105	106	108	110	111	112	60	60	61	62	63	64	64
	90th	117	118	120	122	124	125	126	75	75	76	77	78	79	79
	95th	121	122	124	126	128	129	130	79	79	80	81	82	83	83
	99th	128	130	131	133	135	136	137	87	87	88	89	90	91	91
14	50th	106	107	109	111	113	114	115	60	61	62	63	64	65	65
	90th	120	121	123	125	126	128	128	75	76	77	78	79	79	80
	95th	124	125	127	128	130	132	132	80	80	81	82	83	84	84
	99th	131	132	134	136	138	139	140	87	88	89	90	91	92	92
15	50th	109	110	112	113	115	117	117	61	62	63	64	65	66	66
	90th	122	124	125	127	129	130	131	76	77	78	79	80	80	81
	95th	126	127	129	131	133	134	135	81	81	82	83	84	85	85
	99th	134	135	136	138	140	142	142	88	89	90	91	92	93	93
16	50th	111	112	114	116	118	119	120	63	63	64	65	66	67	67
	90th	125	126	128	130	131	133	134	78	78	79	80	81	82	82
	95th	129	130	132	134	135	137	137*	82	83	83	84	85	86	87
	99th	136	137	139	141	143	144	145	90	90	91	92	93	94	94
17	50th	114	115	116	118	120	121	122	65	66	66	67	68	69	70
	90th	127	128	130	132	134	135	136	80	80	81	82	83	84	84
	95th	131	132	134	136	138	139	140	84	85	86	87	87	88	89
	99th	139	140	141	143	145	146	147	92	93	93	94	95	96	97

BP, blood pressure

Appendix VIII: Female adolescents' blood pressure-for-age

Blood Pressure Levels for Girls by Age and Height Percentile

Age (Year)	BP Percentile ↓	Systolic BP (mmHg) ← Percentile of Height →							Diastolic BP (mmHg) ← Percentile of Height →						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	50th	83	84	85	86	88	89	90	38	39	39	40	41	41	42
	90th	97	97	98	100	101	102	103	52	53	53	54	55	55	56
	95th	100	101	102	104	105	106	107	56	57	57	58	59	59	60
	99th	108	108	109	111	112	113	114	64	64	65	65	66	67	67
2	50th	85	85	87	88	89	91	91	43	44	44	45	46	46	47
	90th	98	99	100	101	103	104	105	57	58	58	59	60	61	61
	95th	102	103	104	105	107	108	109	61	62	62	63	64	65	65
	99th	109	110	111	112	114	115	116	69	69	70	70	71	72	72
3	50th	86	87	88	89	91	92	93	47	48	48	49	50	50	51
	90th	100	100	102	103	104	106	106	61	62	62	63	64	64	65
	95th	104	104	105	107	108	109	110	65	66	66	67	68	68	69
	99th	111	111	113	114	115	116	117	73	73	74	74	75	76	76
4	50th	88	88	90	91	92	94	94	50	50	51	52	52	53	54
	90th	101	102	103	104	106	107	108	64	64	65	66	67	67	68
	95th	105	106	107	108	110	111	112	68	68	69	70	71	71	72
	99th	112	113	114	115	117	118	119	76	76	76	77	78	79	79
5	50th	89	90	91	93	94	95	96	52	53	53	54	55	55	56
	90th	103	103	105	106	107	109	109	66	67	67	68	69	69	70
	95th	107	107	108	110	111	112	113	70	71	71	72	73	73	74
	99th	114	114	116	117	118	120	120	78	78	79	79	80	81	81
6	50th	91	92	93	94	96	97	98	54	54	55	56	56	57	58
	90th	104	105	106	108	109	110	111	68	68	69	70	70	71	72
	95th	108	109	110	111	113	114	115	72	72	73	74	74	75	76
	99th	115	116	117	119	120	121	122	80	80	80	81	82	83	83
7	50th	93	93	95	96	97	99	99	55	56	56	57	58	58	59
	90th	106	107	108	109	111	112	113	69	70	70	71	72	72	73
	95th	110	111	112	113	115	116	116	73	74	74	75	76	76	77
	99th	117	118	119	120	122	123	124	81	81	82	82	83	84	84
8	50th	95	95	96	98	99	100	101	57	57	57	58	59	60	60
	90th	108	109	110	111	113	114	114	71	71	71	72	73	74	74
	95th	112	112	114	115	116	118	118	75	75	75	76	77	78	78
	99th	119	120	121	122	123	125	125	82	82	83	83	84	85	86
9	50th	96	97	98	100	101	102	103	58	58	58	59	60	61	61
	90th	110	110	112	113	114	116	116	72	72	72	73	74	75	75
	95th	114	114	115	117	118	119	120	76	76	76	77	78	79	79
	99th	121	121	123	124	125	127	127	83	83	84	84	85	86	87
10	50th	98	99	100	102	103	104	105	59	59	59	60	61	62	62
	90th	112	112	114	115	116	118	118	73	73	73	74	75	76	76
	95th	116	116	117	119	120	121	122	77	77	77	78	79	80	80
	99th	123	123	125	126	127	129	129	84	84	85	86	86	87	88

Appendix VIII: Female adolescents' blood pressure-for-age (cont.)

Blood Pressure Levels for Girls by Age and Height Percentile (Continued)

Age (Year)	BP Percentile ↓	Systolic BP (mmHg) ← Percentile of Height →							Diastolic BP (mmHg) ← Percentile of Height →						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
11	50th	100	101	102	103	105	106	107	60	60	60	61	62	63	63
	90th	114	114	116	117	118	119	120	74	74	74	75	76	77	77
	95th	118	118	119	121	122	123	124	78	78	78	79	80	81	81
	99th	125	125	126	128	129	130	131	85	85	86	87	87	88	89
12	50th	102	103	104	105	107	108	109	61	61	61	62	63	64	64
	90th	116	116	117	119	120	121	122	75	75	75	76	77	78	78
	95th	119	120	121	123	124	125	126	79	79	79	80	81	82	82
	99th	127	127	128	130	131	132	133	86	86	87	88	88	89	90
13	50th	104	105	106	107	109	110	110	62	62	62	63	64	65	65
	90th	117	118	119	121	122	123	124	76	76	76	77	78	79	79
	95th	121	122	123	124	126	127	128	80	80	80	81	82	83	83
	99th	128	129	130	132	133	134	135	87	87	88	89	89	90	91
14	50th	106	106	107	109	110	111	112	63	63	63	64	65	66	66
	90th	119	120	121	122	124	125	125	77	77	77	78	79	80	80
	95th	123	123	125	126	127	129	129	81	81	81	82	83	84	84
	99th	130	131	132	133	135	136	136	88	88	89	90	90	91	92
15	50th	107	108	109	110	111	113	113	64	64	64	65	66	67	67
	90th	120	121	122	123	125	126	127	78	78	78	79	80	81	81
	95th	124	125	126	127	129	130	131	82	82	82	83	84	85	85
	99th	131	132	133	134	136	137	138	89	89	90	91	91	92	93
16	50th	108	108	110	111	112	114	114	64	64	65	66	66	67	68
	90th	121	122	123	124	126	127	128	78	78	79	80	81	81	82
	95th	125	126	127	128	130	131	132	82	82	83	84	85	85	86
	99th	132	133	134	135	137	138	139	90	90	90	91	92	93	93
17	50th	108	109	110	111	113	114	115	64	65	65	66	67	67	68
	90th	122	122	123	125	126	127	128	78	79	79	80	81	81	82
	95th	125	126	127	129	130	131	132	82	83	83	84	85	85	86
	99th	133	133	134	136	137	138	139	90	90	91	91	92	93	93

BP, blood pressure

Appendix IX: Ethics Clearance

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA
DEPARTMENT OF HEALTH AND SOCIAL DEVELOPMENT

ETHICS COMMITTEE
CLEARANCE CERTIFICATE
UNIVERSITY OF LIMPOPO
Polokwane/Mankweng Hospital Complex

UNIVERSITY OF LIMPOPO

PROJECT NUMBER: 040/2008

TITLE: Risk factors for cardiovascular disease in rural subjects age 15 – 20 year old residing in Dikgale Demographic Surveillance System (DDSS) Site, Limpopo Province, South Africa

RESEARCHER: Nkosinathi Terrence Phoku

ALL PARTICIPANTS: N/A

Department of Medical Science,
School of Pathology, University of Limpopo

Supervisor: prof. M. Alberts
Co supervisor Mr. S.S. Choma

Date considered:
Decision of Committee: Recommended for Approval

Date: 11.10.2008

Prof. A.J. Mbokazi
24/10/08
Chairman of Pietersburg Mankweng
Hospital Complex Ethics Committee

Note: The budget for research has to be considered separately. Ethics Committee is not providing any funds for projects.