The Effects of Cholesterol and Obesity on Carotid Intima Media Thickness in a Population of African Ancestry

Moekanyi Jeffrey Sibiya

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, for the degree of Master of Science in Medicine.

Johannesburg, 2012

Abstract

Cholesterol is an important determinant of atherogenesis. However, populations of African ancestry have low total and LDL cholesterol concentrations (anti-atherogenic), but elevated triglyceride concentrations and low HDL concentrations (pro-atherogenic). The role of circulating lipids in atheroma formation in groups of African descent is uncertain. Therefore, in the current study I evaluated whether circulating lipids are independently associated with carotid intima media thickness (C-IMT), a surrogate marker of atheroma, in an urban developing community of African ancestry in SOWETO South Africa. 430 participants were randomly selected. C-IMT was determined from Doppler images of the carotid artery using a SonoSite (SonoCalc^{TM} IMT) version 3.4 device. In bivariate analysis total cholesterol, total:HDL cholesterol ratio, LDL cholesterol and triglyceride concentrations were all associated with C-IMT ($r=0.24$ to $r=0.26$, p-values <0.0001) as were all indices of obesity ($r=0.16$ to 0.30, $p<0.001$). However, in multivariate models which included adjustments for age, clinic systolic blood pressure, diabetes mellitus, smoking, treatment for hypertension, regular alcohol intake, heart rate and postmenopausal status (in women) neither the lipid variables nor the indices of obesity remained associated with C-IMT (total cholesterol $r=-0.01$, $p=0.884$; LDL $r=0.02$, $p=0.682$; TRGL $r=-0.01$, $p=0.832$; HDL $r=-0.04$, $p=0.371$; total:HDL cholesterol ratio $r=0.02$, $p=684$). In multivariate regression analysis only age ($p<0.0001$) and gender ($p< 0.05$) were independently associated with C-IMT.

In conclusion, the current study shows that in an urban developing community of African ancestry with low cholesterol concentrations and a high prevalence of obesity there is no independent relationship between either circulating lipid concentrations and C-IMT or measures of obesity and C-IMT. However, age and gender independently predicted C-IMT in this population.

Table of contents

Declaration

I declare that this is my own unaided work. It is being submitted for the degree of Master of Science in Medicine in the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. The work contained in this dissertation has not been submitted for any degree or examination in this University, or any other University.

.....

Moekanyi Jeffrey Sibiya

.....on this.....14th..... day of .....June....., 2012

I certify that the studies contained in this dissertation have the approval of the Committee for Research in Human Studies of the University of the Witwatersrand, Johannesburg. The ethics approval numbers are M02-04-72 and renewed as M07-04-69

.....

Moekanyi Jeffrey Sibiya

..on this.....14th.....day of.....June....., 2012

.....

Olebogeng H. Majane (supervisor)

Date.....

.....

Angela J. Woodiwiss (supervisor)

Date.....

Dedication:
For my parents,
Samuel and Iris
Sibiya

Publications and presentations

Data presented in this dissertation have been presented in the form of oral presentations at the 39th Annual Conference of the Physiology Society of Southern Africa held at the University of the Western Cape in Cape Town, September 2011. The titles of the presentations were:

Moekanyi J Sibiya, Angela J Woodiwiss, Gavin R Norton & Olebogeng HI Majane. The impact of circulating cholesterol concentrations on carotid intima media thickness in an urban, developing community of African ancestry.

Olebogeng HI Majane, Moekanyi J Sibiya , Lebogang S Metsing, Gavin R Norton & Angela J Woodiwiss. Associations of indices of adiposity with carotid intima media thickness in people of African descent.

Appendix 1

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Woodiwiss/Norton

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER MO70469**
PROJECT	Gene Candidates As Determinants of Blood Pressure and Intermediary Phenotypes in Pathogenesis of Hypertension in Black S Africans
INVESTIGATORS	Profs A/G Woodiwiss/Norton
DEPARTMENT	School of Physiology
DATE CONSIDERED	07.05.09
DECISION OF THE COMMITTEE*	Approved unconditionally (refer M020472)

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07.05.09 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Woodiwiss A Prof

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Woodiwiss/Norton et al

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M02-04-72

PROJECT Gene Candidates As Determinants of Blood Pressure And Intermediary Phenotypes In Pathogenesis of Hypertension In Black South Africans

INVESTIGATORS Prof's AJ/G et al Woodiwiss/Norton et al

DEPARTMENT School of Physiology, Wits Medical School

DATE CONSIDERED 02-04-26

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON-JONES
HREC (MEDICAL)
2007-05-09
JOHANNESBURG

This clearance is valid and within the Wits 5-year validity

DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 02-05-14 **CHAIRMAN**.....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof AJ Woodiwiss
Dept of School of Physiology, Wits Medical School

Works2\lain0015\HumEth97.wdb\M 02-04-72

==========

DECLARATION OF INVESTIGATOR(S).

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Acknowledgements

I would like to thank my supervisors, Olebogeng Majane and Angela Woodiwiss for their unwavering support, patience and dedication that has made this degree possible, and also Gavin Norton for his academic guidance. In addition, I would like to thank Nomonde Molebatsi, Nkele Maseko and Mthuthuzeli Kiviet for their Technical support.

The current study was funded by grants awarded to Dr. Olebogeng Majane and Prof. Angela Woodiwiss from the South African National Research Foundation. I would also like to thank TATA Africa for funding me in my personal capacity to be able to execute my studies efficiently. The Cardiovascular Pathophysiology and Genomics Research Unit, the School of Physiology and Faculty of Health Sciences of the University of the Witwatersrand also supported these studies.

My contributions to data collection and analysis

I collected the carotid intima media thickness data and the anthropometric data with the assistance of a qualified nurse. The blood samples were sent to an accredited laboratory (Contract laboratory services, Braamfontein) for analysis. I analysed all the data by myself in consultation with my supervisors.

List of Abbreviations

BMI	body mass index
BP	blood pressure
bpm	beats per minute
CCA	common carotid artery
CHD	coronary heart disease
cm	centimeters
CVD	cardiovascular disease
C-IMT	carotid intima media thickness
DM	diabetes mellitus
HbA1C	glycated haemoglobin
HDL cholesterol	high density lipoprotein cholesterol
IMT	intima media thickness
LDL cholesterol	low density lipoprotein cholesterol
MAP	mean arterial pressure
mg/dL	milligrams per decilitre
mm	millimeters
mm Hg	millimeters of mercury
mmol/l	millimoles per litre
$m.s^{-1}$	metres per second
NEFA	nonesterified fatty acids
NIH	National Institutes of Health
oxLDL	oxidatively modified low density lipoprotein cholesterol
PP	Pulse pressure

PWV	pulse wave velocity
RAAS	renin-angiotensin-aldosterone system
SAS	statistical analysis software
SBP	systolic blood pressure
WC	waist circumference
WHR	waist-hip ratio
yr	year

List of tables

Page number

List of figures

Page number

Preface

The high incidence of cardiovascular (CVD) morbidity and mortality has reached epidemic proportions worldwide. It is estimated that in South Africa, the age standardized CVD mortality rates are approximately 350/100,000, which has been explained by the transition from traditional African lifestyles to a more westernized behavior. Changes in large artery wall thickness, as measured by increases in the common carotid intima media thickness (C-IMT), have been shown to predict cardiovascular events such as myocardial infarction and stroke, and to act as a surrogate marker of atherosclerosis especially in patients who are asymptomatic. A prospective study showed that every 0.1mm increase in C-IMT had the potential to increase coronary events by approximately 30%.

A number of cardiovascular risk factors including hypercholesterolemia, obesity, hypertension, diabetes mellitus and smoking have been associated with C-IMT. However, as obesity and increased cholesterol concentrations often occur together, the question arises as to whether obesity or cholesterol concentrations have effects on C-IMT independent of each other. In this regard, although black Africans and African Americans are reported to have lower cholesterol concentrations than Caucasians, C-IMT values are reported to be higher in African Americans compared to Caucasians. However, black Africans and African Americans have a higher prevalence of obesity than Caucasians; hence it is possible that obesity may impact on C-IMT irrespective of cholesterol concentrations. However, to-date the data are unclear on this issue. One of the difficulties in addressing this issue is the strong association between obesity and lipid profiles in most populations. However, a population of black Africans living in South Africa was noted to have a high degree of adiposity (66%), but normal to low lipid levels. This population is therefore ideal to address the possible impact of obesity on C-IMT independent of the effects of lipids.

Hence, in the current study I aimed to determine the association of measures of obesity, cholesterol concentrations and other conventional cardiovascular risk factors with C-IMT in people of African ancestry with a high percentage of obesity and low cholesterol concentrations. In particular I addressed whether indices of obesity independently predict C-IMT and/or measures of lipid profile independently predict C-IMT in the whole population as well as in gender-specific groups. In chapter 1, I therefore discuss the measurement of C-IMT, mechanisms of changes in C-IMT and the association between various cardiovascular risk factors with C-IMT particularly focusing on cholesterol and obesity or the potential interactive effects thereof. In chapter 2, I describe the methods used to collect the C-IMT, anthropometric and lipid profile data. The results of my study are presented in chapter 3, and then in chapter 4, I discuss my data in comparison to the literature and propose possible mechanisms. The results emanating from this study will go a long way in adding value to the scientific knowledge on prevention, diagnosis and treatment of cardiovascular disease particularly in populations undergoing economic transition.

Chapter 1

Introduction

1.0 Introduction

The global population continues to experience a high rate of mortality and morbidity from cardiovascular disease (CVD) (Lerner and Kannel 1986, Lopez *et al.* 2001, Strong *et al.* 2007). It is estimated that in South Africa the age standardized CVD mortality rates are approximately 350/100,000 (Bradshaw *et al.* 2003) and this can be explained by the transition from traditional African lifestyles to a more westernized behaviour (Hamer *et al.* 2011). Cardiovascular related high mortality rates of 195 people per day were noted in South Africa between the year 1997 and 2004 (Steyn & Fourie, 2007). Large artery changes have been associated with cardiovascular events including strokes (O'Donnell *et al.* 2008). In this regard increases in the common carotid intima media thickness (C-IMT), a measure of arterial wall thickness, has been shown to predict cardiovascular events such as myocardial infarction and stroke (Lorenz *et al.* 2006), and to act as a surrogate marker of atherosclerosis especially in patients who are asymptomatic (Pignoli *et al.* 1986; Veller *et al.* 1993; Ebrahim *et al.* 1999; Belcaro *et al.* 2001; Clement *et al.* 2003; Sharma *et al.* 2009,). Indeed, a prospective study by Raitakari *et al.* 2003 showed that every 0.1mm increase in C-IMT had the potential to increase coronary events by approximately 30%. Furthermore in a large 10-year follow-up of 10000 asymptomatic subjects it was shown that those subjects who were at a higher risk of future cardiovascular events had greater presence of stenosing plaques (Belcaro *et al.* 2001).

A number of cardiovascular risk factors including hypercholesterolemia, obesity, hypertension, diabetes mellitus and smoking have been associated with C-IMT (Connor *et al.* 2009; Ebrahim *et al.* 1999; Sharma *et al.* 2009). The association of cholesterol concentrations with C-IMT is well documented (table 1.1). More recently obesity which is one of the

important risk factors for CVD (Manson *et al.*1995; Bray, 2004), has been shown to be associated with increased C-IMT (table 1.2). However, as obesity and increased cholesterol

Table 1.1 Summary of main studies showing an association between cholesterol concentrations and C-IMT

Authors	Type of study	Age	Methodology	Findings
Salonen & Salonen 1991	Population based sample of 1224 Caucasian Eastern Finnish men.	42-60 yrs	ATL UM4 duplex ultrasound system with a 10 MHz transducer in B-mode & 5 MHz transducer in pulsed Doppler mode.	Serum LDL cholesterol concentration associated with C-IMT (beta = 0.125, p <0.0001) in multivariate regression analysis (adjustors=age, ambulatory pulse pressure, smoking, history of ischemic heart disease, systolic BP, diabetes mellitus, Eastern Finish origin, serum copper concentration).
Davis *et al.* 2001	Cohort follow up (25 yrs) study of 725 men and women.	33-42 yrs	B mode ultrasound system biosound phase 2.	Young adult and current LDL cholesterol concentrations associated with increased C-IMT in both men (OR=1.39 & 2.00 respectively) and women (OR=1.54 & 1.74 respectively) in multivariate analysis (adjustor=age).
Baldassarre *et al.* 2002	Cohort of 559 Italian men & women with hyperlipidaemia.	Mean age 53 yrs	Ultrasonography using an 8-MHz transducer.	LDL cholesterol concentration associated with increased C-IMT (beta= 0.00075, p= 0.0024) in multivariate regression analysis (adjustor=age, systolic BP, glucose).
Urbina *et al.* 2002	Cohort of 518 men and women from Bogalusa Heart Study (65% Caucasian)	20-38 yrs	Ultrasound with a 7.5 MHz linear array transducer.	LDL and HDL cholesterol concentrations associated with C-IMT in multivariate regression analysis (partial r=0.13 & 0.09 respectively) (adjustors=age, race, gender, BMI, waist circumference, systolic BP, diastolic BP, log triglycerides, log insulin, glucose).
Raitakari *et al.* 2003	Cohort follow up (21 yrs) study of 2229 Caucasian men and women.	24-39 yrs	Ultrasound mainframes using 13.0 MHZ linear array transducer.	Childhood LDL cholesterol concentration (beta=0.003, p=0.001) associated with adult C-IMT in multivariate regression analysis (adjustors=age, sex).
Lorenz *et al.* 2006	5056 male German participants of the Carotid Atherosclerosis Progression Study	19-90 yrs	Ultrasound with a 7.5 to 10.0 MHz linear array transducer.	LDL cholesterol associated with increased C-IMT (p<0.0001) in univariate analysis.
Sarmento *et al.* 2009	18 women undergoing bariatric surgery	Mean age 44 yrs	Ultrasound with a 7.5 to 10.0 MHz linear array transducer.	Decrease in triglyceride concentration associated with decrease in C-IMT.
Paul *et al.* 2011	Cohort of 518 men and women from Bogalusa Heart Study (71% Caucasian)	24-43 yrs	Ultrasound with a 7.5 MHz linear array Transducer.	Total:HDL cholesterol ratio associated with C-IMT in multivariate regression analysis (partial r=0.18, p<0.05) (adjustors=age, race, gender, BMI, waist circumference, systolic BP, diastolic BP, smoking LDL cholesterol)

Table 1.2 Summary of main studies showing an association between obesity and C-IMT

Authors	Type of study	Age	Methodology	Findings
Ciccone *et al.* 2001	Cohort of 120 healthy Italian men and women.	18-45 yrs	Ultrasonography using an 8-MHz transducer.	Plasma leptin and BMI associated with C-IMT ($p < 0.0001$ & $p < 0.01$ respectively) in multivariate regression analysis (adjustors=age, gender, rate constant for glucose disappearance, systolic BP, log triglycerides, total cholesterol concentration, HDL cholesterol concentration).
de Michele *et al.* 2002	Cohort of 310 Italian women.	30-69 yrs	B mode ultrasound system biosound 2000 II SA.	BMI and WHR associated with increased C-IMT ($p < 0.005$ & $p < 0.01$ respectively) in multivariate analysis (adjustor=age, systolic BP, LDL cholesterol concentration, triglyceride concentration, diastolic BP, smoking, insulin concentration).
Lo *et al.* 2006	Cohort of 100 healthy women (35% Caucasian, 45% African American).	24-59 yrs	Ultrasonography using a 7.5 MHz phased-array transducer.	Subcutaneous fat (CT scan) associated with C-IMT ($p = 0.01$) in multivariate regression analysis (adjustor=age, race, smoking, systolic BP, diastolic BP, BMI, visceral fat area, cholesterol concentration, LDL cholesterol concentration, HDL cholesterol concentration, triglyceride concentration, C-reactive protein, insulin resistance).
Naya *et al.* 2008	Cohort of 241 Japanese men and women .	18-62 yrs	B mode ultrasound with a 5-10 MHz annular array transducer.	BMI associated with C-IMT in multivariate regression analysis ($p < 0.0001$) (adjustors=smoking, systolic BP, insulin, glucose).
Koskinen *et al.* 2009	Cohort follow up (6 yrs) study of 1809 Finish men and women.	Mean age 32 yrs	Ultrasound mainframes using 13.0 MHZ linear array transducer.	Waist circumference associated with C-IMT in multivariate regression analysis ($p < 0.0001$ (adjustors=age, sex, systolic BP, LDL cholesterol concentration, HDL cholestero concentration, log insulin, family history of coronary artery disease).
Maher *et al.* 2009	Cohort of 100 healthy Irish men and women,	Mean age 41 yrs	Ultrasound with a 7 MHz linear array transducer.	BMI and waist-to-height ration associated with increased C-IMT ($p < 0.01$) in multivariate regression analysis (adjustors=age, gender insulin resistance, HDL cholesterol concentration, triglyceride concentration, systolic BP).
Skilton *et al.* 2009	Cohort of 175 Australian men and women	21-83 yrs	Ultrasound with a 8 MHz linear array transducer.	BMI associated with C-IMT ($p < 0.001$) in multivariate regression analysis (adjustors=age, gender, smoking, diabetes mellitus, log triglyceride concentration, HDL cholesterol concentration, LDL cholesterol concentration, systolic BP).
Soliman *et al.* 2010	Cohort of 996 men and women from MESA Study (21% African American)	45-84 yrs	Logiq 700 ultrasound machine (no details of transducer given).	BMI and waist circumference associated with C-IMT in multivariate regression analysis ($p = 0.001$ & $p < 0.0001$ respectively) (adjustors=age, race, hypertension, diabetes mellitus, smoking, cholesterol concentration). Analyses done in men and women separately

concentrations often occur together, the question arises as to whether obesity or cholesterol concentrations have effects on C-IMT independent of each other. In this regard, it is interesting that black Africans (Connor *et al.* 2009) and African Americans (Urbina *et al.* 2002; Paul *et al.* 2011) have lower cholesterol concentrations and increased obesity than Caucasians and yet C-IMTs have been reported to be higher in African Americans compared to Caucasians (Howard *et al.* 1993; Arnett *et al.* 1996; D'Augostino *et al.* 1996; Urbina *et al.* 2002; Paul *et al.* 2011). This data would point toward an effect of obesity on C-IMT irrespective of cholesterol concentration. Indeed, some studies have reported effects of obesity on C-IMT independent of cholesterol concentrations (using multivariate regression models) (Ciccone *et al.* 2001; de Michele *et al.* 2002; Lo *et al.* 2006; Koskinen *et al.* 2009; Skilton *et al.* 2009; Soliman et al. 2010). However, this data is not clear, in that some studies did not adjust for cholesterol concentrations (Naya *et al.* 2008), some report that the relationship with waist circumference was no longer evident after adjustments for lipid concentrations (Hassinen *et al.* 2007), and others report an interactive effect (Lakka *et al.* 2001). Moreover in a weight reduction study, although a reduction in C-IMT was noted, this was associated with decreases in triglyceride concentrations (Sarmento *et al.* 2009). One of the difficulties in addressing this issue is the strong association between obesity and lipid profiles (Lakka *et al.* 2001; de Michele *et al.* 2002; Hassinen *et al.* 2007). However, a population of black Africans living in South Africa was noted to have a high degree of adiposity (66%) (Steyn 2001; Majane *et al.* 2007; Norton *et al.* 2009; Maseko *et al.* 2011[a]), but normal to low lipid levels (Maseko *et al.* 2011[b]). This population is therefore ideal to address the possible impact of obesity on C-IMT independent of the effects of lipids.

Hence, for my MSc I aimed to determine the association of measures of obesity, cholesterol concentrations and other conventional cardiovascular risk factors with C-IMT in people of

African ancestry with a high percentage of obesity and low cholesterol concentrations. In this chapter of my MSc I will therefore discuss the measurement of C-IMT, mechanisms of changes in C-IMT and the association between various cardiovascular risk factors with C-IMT particularly focusing on cholesterol and obesity or the potential interactive effects thereof.

1.1 Non-invasive measurements of the carotid artery

The non-invasive measurement of the intima-media thickness of the carotid arterial walls is done by ultrasound imaging of carotid arteries (figure 1.1) (Poredos, 2004). In the assessment of cardiovascular risk, ultrasound imaging of the carotid arteries to measure C-IMT is particularly useful in the early detection of target organ damage and atherosclerotic lesions (Pignoli *et al.* 1986; Veller *et al.* 1993; Belcaro *et al.* 2001). Although some authors have measured the maximum IMT which extends to the external elastic membrane (Toubol *et al.* 1992); in study designs where only the common carotid artery is measured, investigators have come to prefer the measurement of the mean carotid IMT (Polak, 2009; Bots *et al.* 1997; Toubol *et al.* 2007). This is the average of the mean IMT measured in the common carotid arteries (Polak, 2009). Both the near and the far wall of the common carotid arteries were initially included in this measurement, however the current trend is to limit the C-IMT measurement to the far wall of the common carotid artery based on the physical constraints of ultrasound imaging transducers (Bots *et al.* 1997; Taylor *et al.* 2002).

We reported on C-IMT values of the left carotid artery because we were more interested in the association of C-IMT and blood biochemical indices such as LDL cholesterol. The right

C-IMT primarily correlates with hemodynamic parameters (mean velocity, pulsatility index, and ratio of the maximum to minimum velocity), while the left C-IMT correlates better with

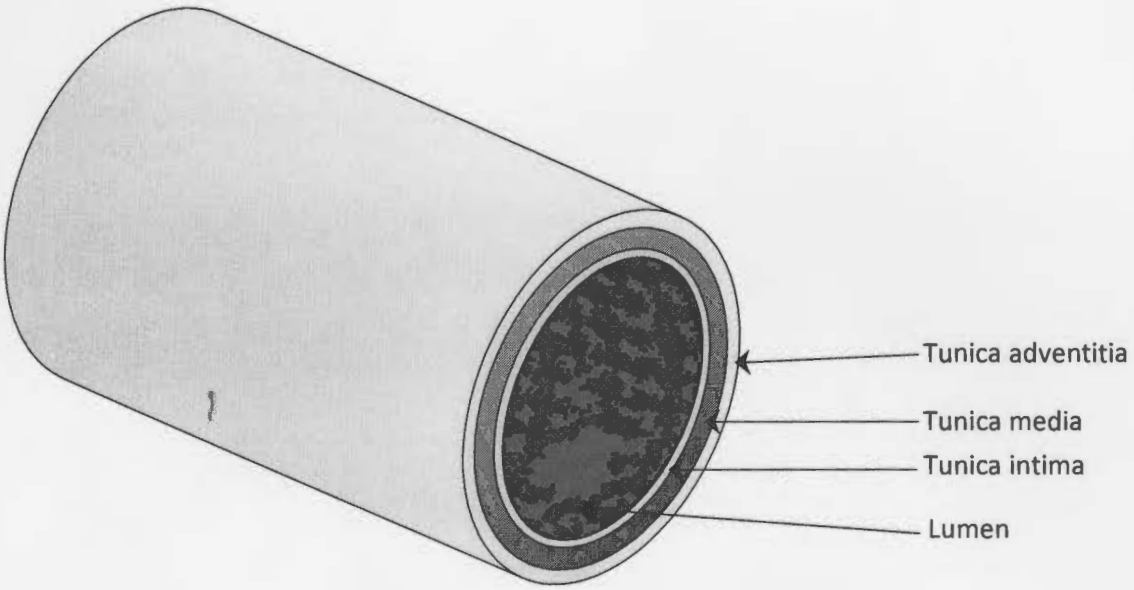

Figure 1.1 A diagrammatic representation of a cross-section of a normal carotid artery. The intima-media thickness is the distance between the lumen-intima interface and the media-adventitia interface.

blood biochemical indices (total cholesterol, LDL cholesterol and blood glucose level) (Luo *et al.* 2011).

Despite the increasing clinical use of C-IMT in the assessment of cardiovascular risk, there are no standardized guidelines at this time that define the thresholds for low, moderate, or high risk (Cobble & Bale, 2010). Currently the American Society of Echocardiography (ASE) defines high risk as those patients with a C-IMT above the 75th percentile for their age, sex, and race (Stein *et al.* 2008). In the ASE consensus statement C-IMT was considered increased if it was in the highest quartile (i.e. $\geq$ 75th percentile for a person's age, sex and race (African American and Caucasian), and nomograms of C-IMT values from large, population-based studies in North America and Europe are provided for reference (Stein *et al.* 2008, Howard *et al.* 1993, Tzou *et al.* 2007). Importantly, the measurement of C-IMT is a useful test for refining CVD risk assessment in patients, provided they do not already have conditions that indicate high CVD risk (such as established CVD or risk equivalent conditions) (Stein *et al.* 2008).

In addition to the measurement of C-IMT, more advanced vascular disease (such as occurs in patients with high CVD risk) is identified by the presence of carotid plaque which is defined as a focal thickening increase of $\geq$1mm (McQuillan *et al.* 1999). In pathological terms atherosclerotic plaque is defined as the thickness of the wall from the lumen to the internal elastic membrane. Carotid plaque formation tends to be greater in people with a thicker C-IMT in cardiovascular disease (Bonithon-kopp *et al.* 1996), implying that those with an IMT at or above the 75th percentile would most likely have a plaque formation. However, as the population that I assessed did not have high CVD risk (only 4% had established CVD) (Norton *et al.* 2012), I did not assess carotid plaque in the current study.

1.2 Effects of cholesterol on arterial wall thickness

One of the primary factors responsible for increases in arterial wall thickness is an increase in low density lipoprotein (LDL) cholesterol (Poredos 2004; Beckett *et al.* 2000), which plays an important role in the initiation and progression of atherosclerosis (Naito *et al.* 1994). Briefly, an increase in LDL cholesterol initiates the process of atherosclerosis which begins with LDL cholesterol moving across the plasma membrane and into the intima of the artery (Jia *et al.* 2009). The LDL cholesterol is then oxidized and engulfed by macrophages (transformed mast cells). These cells then undergo apoptosis and release the fat which results in the formation of a large fat droplet in the intima of the artery. The smooth muscle cells in the media of the artery proliferate and migrate to the intima, where they are transformed into fibroblasts. The fibroblasts are responsible for the synthesis of collagen and hence result in increased fibrosis within the intima. Ultimately, the accumulation of fat and fibrosis leads to leads to the thickening of the innermost layer of the arterial wall and hence an increase in C-IMT (figure 1.2). An increase in C-IMT will result in lumen narrowing and hence a reduction in blood flow. In the more advanced stages of atherosclerosis, plaque formation and endothelial dysfunction occur (Stocker *et al.* 2004; Tan *et al.* 2009), which results in further narrowing of the arterial lumen and a reduction in blood flow.

The potential mechanisms and atherogenic effects of oxidatively modified low density lipoprotein cholesterol (oxLDL) during the formation of atheroma include chemotactic activity, which facilitates recruitment of blood monocytes; inhibition of the migration of macrophages from the arterial wall back to the plasma; enhanced uptake of macrophages through the scavenger receptors, leading to the formation of foam cells; and cytotoxicity. These processes lead to the accumulation of LDL in the arterial wall which then consecutively causes endothelial denudation at a later stage (Steinberg, 1989).

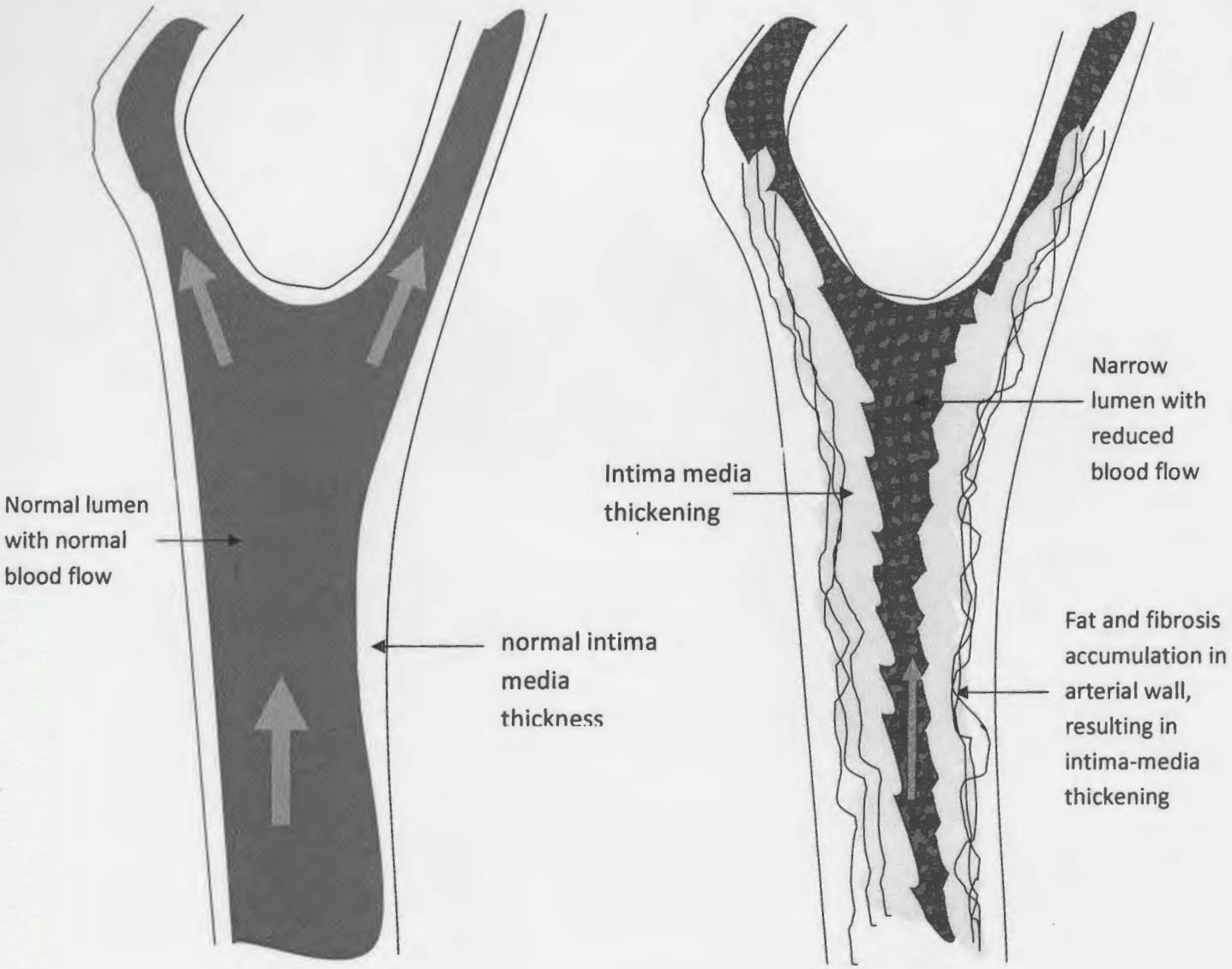

Figure 1.2 A schematic representation showing changes in the common carotid-intima media thickness that occur due to the increase in LDL cholesterol and the resulting accumulation of fat and fibrosis within the intima and media.

In addition to increases in LDL cholesterol being implicated in atherosclerosis, a decrease in high density lipoprotein (HDL) cholesterol also increases atherosclerotic risk (Castelli *et al* 1989; Beckett *et al.* 2000). Physiologically high HDL cholesterol and low LDL cholesterol concentrations are needed. LDL cholesterol is considered as a ''bad'' cholesterol as when it accumulates or increases in the artery it begins to obstruct the artery as described above. In comparison, HDL cholesterol is considered as a "good" cholesterol as it carries approximately 20% of the total plasma cholesterol (Castelli *et al.* 1997). Hence its physiological role in the body is to remove LDL cholesterol from the blood stream and peripheral tissues thus transporting it to the liver where it is catabolized and excreted from the body (Miller & Miller, 1975). It is important to note that the concentration of HDL cholesterol is inversely proportional to the risk of developing CVD (Third report of the National Cholesterol Education program (NCEP), 2001). Ideally there should be a higher concentration of HDL cholesterol as opposed to LDL cholesterol in the blood. Furthermore, this inverse association between HDL and LDL cholesterol is mediated by reverse cholesterol transport which is a transport system caused by a collection of physiological events that transport cholesterol from peripheral tissues to the liver for further metabolism or for excretion from the body (Lichtenstein *et al.* 2006).

Triglycerides are also important in CVD because they have been found to contribute to dyslipidemia (Bamba & Rader 2007). In dyslipidemia triglyceride levels are abnormally high, however if they become extremely high they may cause the liver or spleen to enlarge because many nonesterified fatty acids (NEFA) delivered to the liver are re-esterified into triglycerides (Ginsberg *et al.* 2006). Moreover, although the mechanisms are not known, increased triglyceride concentrations are often associated with decreased HDL cholesterol concentrations (Wilson & Grundy 2003; Gaziano *et al.* 1997). Most importantly, the risk of

atherosclerosis increases as total cholesterol and LDL cholesterol increases even if the levels are not high enough to be considered dyslipidemia.

Although there is no doubt that cholesterol is an important factor in determining C-IMT, populations of African ancestry have relatively low total cholesterol concentrations (anti-atherogenic) (Omran, 2005); yet evidence from our laboratory shows that people of African ancestry have elevated triglyceride concentrations (unpublished data). Bearing in mind the inverse relationship between triglycerides and HDL cholesterol concentrations (Wilson & Grundy 2003; Gaziano *et al.* 1997), especially in the presence of metabolic syndrome (combination of obesity, dyslipidaemia, hypertension, and diabetes mellitus) (Wilson & Grundy 2003); and that there is a high incidence of obesity and hypertension in populations of African ancestry (Steyn 2001; Majane *et al.* 2007; Norton *et al.* 2009; Maseko *et al.*2011[a]), the question arises as to whether factors such as triglycerides, HDL cholesterol, obesity and hypertension contribute towards increases in C-IMT in populations of African ancestry.

1.3 Effects of high blood pressure on arterial wall thickness

Increased systolic blood pressure (SBP) causes structural arterial wall changes which lead to an increased C-IMT. The potential explanation is that high blood pressure causes thickening of large elastic and muscular fibers; as well as remodeling (hypertrophy) of small muscular arteries thus resulting in increased wall to lumen ratio (Thom, 1997). The above mentioned changes cause the C-IMT to increase due to the increase in collagen fibers which may lead to arterial stiffening. In addition, high blood pressure is known to enhance oxidation of LDL cholesterol and decrease endothelial function. Studies in people of African ancestry living in South Africa show a high prevalence of hypertension in this population group (Steyn 2001;

Maseko *et al.* 2006 Norton *et al.* 2009; Maseko *et al.* 2011[a]). More alarming is that about 50% of these hypertensives are not receiving antihypertensive treatment (Maseko *et al.* 2011[b]).

The main contributing factor for untreated hypertension in people of African ancestry could be the lack of awareness of hypertensive status and lack of regular blood pressure check-ups. Results from the demographic and health survey conducted on South Africans in 1998 show that the prevalence rate for hypertension was 11% for men and 14% for women in the whole South African population (Steyn, 2001). With the cut-off point for hypertension placed at 160/95 mmHg it was noted in other studies on various population groups that women had a higher rate of awareness amongst all the population groups as compared to men (Asians, Whites and Africans); however when observing specifically in people of African ancestry one notes that women have a rate of 64% and men 32%. When compared to other population groups (Asians [women 85%] and whites [women 77%, men 64%]) people of African ancestry had the lowest awareness rate overall (Steyn, 2001). Hence it is likely that similar to other populations (Salonen & Salonen 1991; Ciccone *et al.* 2001; Davis *et al.* 2001; Lo *et al.* 2006; Naya *et al.* 2008; Koskinen *et al.* 2009; Maher *et al.* 2009), increases in blood pressure are associated with increases in C-IMT. Hence, in my study I did not focus on the impact of hypertension on C-IMT. However, populations of African ancestry are characterized by a high incidence of obesity especially central obesity (Majane *et al.* 2007), which may or may not impact on C-IMT in the absence of increased lipid concentrations (see section1.0 above). I will therefore now discuss how obesity could affect the structure of the vasculature.

1.4 Effects of obesity on arterial wall thickness

The prevalence of obesity in South Africa has increased dramatically over the past few years. Indeed, studies report approximately 60% of populations of African ancestry to be

overweight or obese (Steyn *et al.* 2005; Majane *et al.* 2007; Norton *et al.* 2009). With regards to the possible impact of obesity on C-IMT, some studies have reported an association between obesity and C-IMT which is independent of blood pressure (BP) and cholesterol concentrations (Ciccone *et al.* 2001; de Michele *et al.* 2002; Lo *et al.* 2006; Koskinen *et al.* 2009; Maher *et al.* 2009; Skilton *et al.* 2009, see table 1.2). The mechanisms of the effects of obesity on C-IMT are not known; but C-IMT is associated with increases in leptin concentration due to increases in obesity (Ciccone *et al.* 2001). However, although in elderly women increases in C-IMT over a 12 year period were associated with increases in waist circumference (WC) and hip circumference (Hassinen *et al.* 2007); this association between C-IMT and WC did not survive adjustments for triglycerides which were elevated in relation to the increases in WC (Hassinen *et al.* 2007). Although, reductions in body weight in obese subjects are reported to be accompanied by decreases in C-IMT, these changes in C-IMT are also accompanied by reductions in BP and cholesterol (Sarmento *et al.* 2009). Hence the independent role of obesity on C-IMT has been questioned. Indeed, in most obese populations cholesterol concentrations are increased. Hence the high prevalence of obesity and yet relatively low cholesterol concentrations in populations of African ancestry provides the ideal opportunity to assess the possible independent role of obesity on C-IMT. Alternatively, obesity and cholesterol may have interactive effects on the vasculature.

1.5 Interactive effects of obesity and cholesterol on arterial wall thickness

Fairly recently, a retrospective study by Kotsis *et al.* 2006 in 3173 participants showed that C-IMT increased progressively across categories of body mass index (BMI) (underweight, normal, overweight and obese) according to National Institutes of Health criteria (NIH guidelines, 1998). After multivariate analyses in obese participants (BMI $\geq$ 30 kg m^{-2}, n=134) only fasting serum glucose was independently associated with C-IMT, although age and

fasting serum cholesterol levels were the next most important factors in the multivariate model. In overweight participants (BMI 25-29.9 kg m^{-2}, n=249), age, male gender, and fasting serum cholesterol levels were independently associated with C-IMT; and in normal weight participants (BMI 18.5-24.9 kg m^{-2}, n=144); 24-hour pulse pressure (PP), duration of hypertension and age were independently associated with C-IMT. (The smaller sample size of obese participants may have resulted in the failure of fasting cholesterol levels to achieve significance). Although no formal interactive analyses were performed in this study (individuals were categorized according to BMI), this data points toward possible interactive effects of obesity and cholesterol on C-IMT. Indeed, a study in young adults showed that increases in WC and LDL cholesterol predicted the progression of C-IMT (Koskinen *et al.* 2009). Although WC increased over the 6 year follow-up period the highest mean value (in those aged 45 years) was 90cm. Bearing in mind that an increased WC in women is ≥ 88cm and in men is ≥ 102cm (Third report of the National Cholesterol Education program 2002), it is unlikely that many of these subjects were obese. Hence, to date no study has assessed in a population with a high degree of adiposity, whether obesity has an interactive effect with cholesterol on C-IMT.

1.6 Possible gender specific effects of obesity and cholesterol on C-IMT

The distribution of fat appears to be important in determining the relationship between measures of obesity and C-IMT in gender specific groups. ''Subcutaneous'' obesity is very common in pre-menopausal women; whereas in both men and post-menopausal women, ''visceral'' obesity is more prevalent (Garaulet *et al.* 2002; Majane *et al.* 2007; Norton *et al.* 2009). The accumulation of fat in peripheral areas (subcutaneous fat) in pre-menopausal women appears to be the consequence of oestrogen effects on visceral adipocytes (Garaulet *et*

al. 2002). However, in women the relationship between subcutaneous abdominal fat area and C-IMT (p=0.010) is stronger than the relationship between visceral abdominal fat area and C-IMT (p=0.092) (Lo *et al.* 2006). BMI does not distinguish between fat mass and fat free-mass since it is based on only body weight and height, and hence it has limitations as an indicator of adiposity (Prentice & Jebb 2001). The stronger associations of cardiovascular disease risk factors with body fatness that have been observed with the subscapular skinfold thickness in comparison to BMI may reflect the importance of fat distribution (Freedman *et al.* 2009). With regard to total cholesterol, men have higher cholesterol concentrations than women. In addition, men have higher LDL cholesterol concentrations and lower HDL concentrations than women (Castelli *et al.* 1989). Chien *et al.* 2008 suggest that the reason men have greater C-IMT than women is because men develop atherosclerosis at an early stage since they are predisposed at birth. This suggests that the increased C-IMT in men could be due to genetic inheritance. Hence cholesterol and obesity, and their possible interactive effects on C-IMT need to be assessed in gender specific groups.

1.6.1 Gender effects on obesity

The prevalence of obesity has reached alarming levels over the last few years which is a major concern for South Africa and the global community at large and it is one of the major health risk factors in the new millennium The etiology of obesity is an imbalance between energy ingested in food and the energy expended. The excess energy is stored in fat cells that enlarge thus increasing in number (Bray, 2004), resulting in the development of obesity. Excess fat results in metabolic changes which may cause risks such as diabetes mellitus, gallbladder disease, hypertension, CVD and some forms of cancer (Bray, 2004). In the Nurses' health study, the risk of death was found to be high in women with a BMI above 29

kg m^{-2} (Manson *et al.* 1995), and in hypertensive men, myocardial wall thickness was associated with plasma leptin, independent of body weight and blood pressure (Paolisso *et al.* 1999). The accumulation of fat in adults is related to gender (Seidell *et al.* 1988), and in this regard obesity is predominant in women of African ancestry when compared to their male counterparts (Puoane *et al.* 2002). Hence the possible impact of obesity on C-IMT needs to be assessed in gender specific groups.

1.6.2 Gender effects on cholesterol

It is estimated that on average women develop heart failure 10 to 15 years later than men (Rossouw, 2001). During the first 30 years of adult life, LDL cholesterol levels are lower in women than in men, and this may be the reason for a delayed onset of coronary heart disease (CHD) in women. Oestrogen benefits women by lowering LDL cholesterol and increasing HDL cholesterol levels and furthermore has antioxidant properties, and improves endothelial function (Mendelsohn and Karas, 1999). Schaefer *et al.* (1994) found that aging was associated with higher levels of LDL cholesterol and apo B levels in both men and women; thus age may cause impairment of LDL cholesterol catabolism and ultimately reduce the activity of LDL receptors in the liver hence causing an increase in LDL cholesterol levels in both men and women. Nevertheless, because of the effects of estrogen in pre-menopausal women, it is important to assess the impact of cholesterol on C-IMT in gender specific groups.

1.7 Is there a physiological explanation as to how age would increase C-IMT? Or is it a biological aging phenomenon

In addition to the independent predictors of C-IMT discussed above, it has been shown that age is the risk factor that is most strongly associated with CIMT (Howard *et al.* 1993; Polak, 2009). At a young age, the rate of increase in C-IMT is more rapid in individuals exposed to high levels of cholesterol because of familial hyperlipidemia (de Groot *et al.* 2004; Wiegman *et al.* 2004). Focusing on the rate at which C-IMT increases with age in the general population, average C-IMT measurements made in groups of individuals spanning an age range of 45 to 85 years show a strong and persistent linear association with age (Polak, 2009). In a previous study (age range: 45 to 64 years) it was found that the rate at which C-IMT increases is approximately 0.010 mm/yr in both men and women (Howard *et al.*1993).

Bearing in mind that the rate of change of C-IMT is in the range of 0.008 to 0.0147 mm/yr, 0.1 mm translates into an expected change over 7 to 10 years (Polak, 2009). Possible explanations for strong association of age with C-IMT is that aging of the arterial wall may cause structural changes such as splitting of elastic fibres, an increase in collagen fibres, smooth muscle (SMC) proliferation, and general stiffening of arteries (Carallo *et al.* 1999; Erusalimsky *et al.* 2009).

Bearing in mind the strong association between age and C-IMT, it is important to note that many of the studies assessing the independent impact of either cholesterol (table 1.1) or obesity (table 1.2) have not assessed the participants over the full adult age range. Most of the studies assessing the impact of cholesterol have only assessed young participants (20-43 yrs of age, table 1.1) (Davis *et al.* 2001; Urbina *et al.* 2002; Raitakari *et al.* 2003; Paul *et al.* 2011) or assessed participants in a narrow age range (40-60 yrs) (Salonen & Salonen 1991). Only Lorenz *et al.* 2006 assessed participants over the full adult age range (19-90

yrs); however this study only performed bivariate analyses (no multivariate analyses were performed). Similarly, most of the studies assessing the impact of obesity have only assessed young participants (table 1.2) (Ciccone *et al.* 2001, 18-45 yrs; Koskinen *et al.* 2009, mean age = 32 yrs; Lo *et al.* 2006, 24-59 yrs), a fairly narrow age range of participants (de Michele *et al.* 2002. 30-69 yrs) or only older participants (Soliman *et al.* 2010). Although in the study by Maher *et al.* 2009, the mean age was 41 years, participants were specifically selected on the basis of normal cholesterol and normal blood pressure values. Naya *et al.* 2008 assessed participants who ranged in age from 18-62 years but they were not able to show a relationship between age and C-IMT. Only one study (Skilton *et al.* 2009) assessed participants over the full adult age range (21-83 yrs); however in this study the maximum BMI was only 28 kg/m^2. Hence, these studies have not adequately addressed the possible independent impact of obesity and/or cholesterol on C-IMT at a population level.

1.8 Ethnic differences in C-IMT

Several studies have shown that the structure and function of the heart in cardiovascular disease varies amongst different ethnic groups. Bearing in mind that C-IMT is a good marker of coronary heart disease (CHD) (Bensen *et al.* 1999, Chien *et al.* 2008), the association between family history of CHD and pre-clinical carotid artery atherosclerosis was investigated. Bensen *et al.* 1999 found no relationship between CHD and C-IMT in African men. However they found an association between CHD and C-IMT in Caucasian men and women. The ethnic difference in the relationship was attributed to the fact that the Caucasians had elevated cholesterol levels, and hence they were more likely to develop atherosclerosis leading to the narrowing of the coronary arteries. However, in contrast to what Bensen *et al.*

(1999) found, several authors have reported that C-IMT is higher in African Americans than in Caucasians (Howard *et al.* 1993; Arnett *et al.* 1996; D'Augostino *et al.* 1996; Urbina *et al.* 2002; Paul *et al.* 2011). Hence, it is imperative to investigate the impact of measures of obesity and cholesterol concentrations on C-IMT in people of African ancestry with a high percentage of obesity and low cholesterol concentrations.

1.9 Problem Statement

To my knowledge there are no studies across the full adult age range that assess the factors associated with increases in C-IMT in populations with low cholesterol concentrations and yet a high incidence of overweight and obesity. To adequately address this issue the possible interactive effects of gender on the relationship between obesity and / or cholesterol on C-IMT need to be assessed.

1.10 Objectives

The primary objectives of the current study were therefore to determine, in an adult population of African ancestry with low cholesterol concentrations and yet a high incidence of overweight and obesity, whether:

1. indices of obesity independently predict C-IMT
2. measures of lipid profile independently predict C-IMT
3. indices of obesity and / or measures of lipid profile independently predict C-IMT in gender-specific groups

4. there is an interactive effect of obesity and cholesterol on C-IMT in the whole population and/or in gender-specific groups.

Chapter 2

Methods

2.0 Methods

2.1 Study Participants

The study was approved by the University of the Witwatersrand Committee for Research in Human Subjects (approval number: M02-04-72 renewed as M07-04-69) and conducted according to the principles outlined in the Declaration of Helsinki. Participants gave informed, written consent. This study is part of the ongoing African Project on Genes in Hypertension which has previously been described (Shiburi *et al* 2006; Maseko *et al*. 2006; Majane *et al*. 2007). South Africans of black African ancestry from nuclear families of the predominant chiefdoms (tribes) of the area (Nguni, Sotho and Venda) with common ancestral backgrounds, were randomly recruited from a metropolitan area of Johannesburg (South Western Townships-SOWETO). Nuclear families were recruited if at least one or two offspring of at least 16 years of age and one or both parents were available for examination.

Of the 538 South Africans of black African ancestry randomly recruited for the measurement of carotid intima media thickness, 430 men and women had complete and high quality common carotid intima media thickness (C-IMT) images, full lipid profile data, and all anthropometric measurements.

2.2 Clinical, demographic and anthropometric measurements

A standardized questionnaire was administered to obtain demographic data and information on each participant's medical history, smoking habits, intake of alcohol, use of medication and menopausal status. The questionnaire was explained to the families at an initial home visit and then subsequently completed in the presence of trained study assistants

at an office visit where questions could be answered. At a second home visit, medications, alcohol consumption and tobacco use in the household was compared against those reported in the questionnaire. Menopause was confirmed with measurement of follicle stimulating hormone concentration (Bayer, Leverkusen, Germany).

Body height and weight were measured with the participants standing and wearing indoor clothes with no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference (WC) was measured at the end of gentle expiration, at the point midway between the lowest rib and the iliac crest with the subject standing. Hip circumference was measured to the nearest 0.1 cm of the girth at the maximum posterior protrusion of the buttocks while the subject was standing upright. Waist-to-hip ratio was then calculated.

Triceps and subscapular skin-fold thickness were determined using a Harpenden skin-fold calipers and the mean of these values was obtained for statistical analyses. The triceps skinfold measurement was made at a point over the triceps muscle, midway between the acromion and olecranon processes on the posterior aspect of the arm by grasping a vertical fold of skin, using the thumb and the forefinger. To identify the subscapular skinfold measurement site, the participant placed his/her left arm behind their back. The measurement site was then marked just below and laterally to the angle of the left shoulder blade. The participant then relaxed the left shoulder and the arm was placed to the side of the body. The caliper was then held at the marked site with the fingers on top, thumb below, and the forefinger on the site of the lower tip of the scapular. Caution was taken for the skinfold to be angled at 45° from the horizontal, in the same direction as the inner boarder of the scapula (medially upwards and laterally downward). The caliper was then used at a 45° angle.

Participants were identified as being overweight if their BMI was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2. Furthermore, abdominal obesity was identified if WC was > 88 cm in women and >102 cm in men.

2.3 Clinic blood pressure (BP)

Trained observers measured brachial artery BP using a mercury sphygmomanometer. The participants were seated and asked to rest for 10 minutes. The observers measured the participants' sitting BP five consecutive times. Systolic and diastolic (phase V) BP were determined to the nearest 2 mm Hg according to the recommendations of the European Society of Hypertension (O'Brien *et al.* 2003). Standard cuffs with an inflatable bladder with a length of 22 cm and a width of 12 cm were used unless the arm circumference exceeded 31 cm, then larger cuffs with a 31 x 15 cm bladder were employed. The five readings were averaged to obtain single systolic, diastolic and mean arterial BP reading.

2.4 Lipid Profile

The concentrations of total and HDL cholesterol were determined at the National Health Laboratory Services (NHLS) situated at Charlotte Maxeke Johannesburg Academic hospital. Venipuncture was performed after a 12-hour fast. Cholesterol concentrations in serum were analyzed with a cholesterol reagent on the Advia chemistry instrument (Siemens, South Africa) (Urbina *et al.*2002). Serum HDL cholesterol concentration was analysed by applying both heparin-calcium precipitation and agarose gel electrophoresis (Urbina *et al* 2009). LDL cholesterol was determined by subtraction using the Friedewald formula.

Friedewald formula: LDL = Total cholesterol - HDL - ([Triglycerides]/5)

The Friedewald formula should not be used under the following conditions:

- When chylomicrons are present.
- When plasma triglyceride concentration exceeds 400 mg/dL (4.52 mmol/l).
- In patients with dysbetalipoproteinemia (type III hyperlipoproteinemia).

In circumstances in which these conditions apply, LDL cholesterol should be measured directly. In the current study no participants had triglyceride concentrations which exceeded 4.52 mmol/l (the maximum triglyceride concentration in the sample was 4.20 mmol/l).

2.5 Other laboratory tests

Standard laboratory tests of renal function, liver function, haematological parameters, blood glucose, and glycated haemoglobin (HbA1C) were performed. Diabetes mellitus or inappropriate blood glucose control were defined as the use of insulin or oral hypoglycaemic agents or an HbA1c (Roche Diagnostics (Mannheim, Germany) >6.14% (Bennet *et al.* 2007).

2.6 Carotid intima-media thickness

Common carotid artery intima media thickness was determined using a SonoSite (SonoCalc™ IMT) version 3.4 through doppler imaging (B-mode ultrasonography) with a high frequency linear probe (HFL38X/13-6 MHz) (figure 2.1). The patient's data and images were captured and saved on the ultrasound system (SonoSite MicroMaxx, USA) then transferred to a personal computer using SiteLink Image Manager for further analysis.

Figure 2.1 The SonoSite (SonoCalc™ IMT) version 3.4 ultrasound MicroMaxx system with linear probe.

2.7 Examination of the carotid intima-media thickness (scanning technique)

The patient's identification data (including participation number, age, gender, ethnicity, blood pressure) was entered on the ultrasound system and a file set up. Examinations were done with the participant lying down using a linear ultrasound probe to capture images. Ultrasound gel (SSEM Mthembu Medical [Pty] Ltd, South Africa) was applied to the probe to act as a conductor, hence enhancing image quality by eliminating air bubbles that could be trapped between the participant's skin and the ultrasound probe. Participants were then asked to turn their heads slightly away from the side on which the measurements were being taken.

The examination was started in a transverse orientation by positioning the probe low in the neck with the orientation marker facing towards the patient's back, down towards the bed. The probe was then moved slowly up the neck until the common carotid artery, thyroid gland and often the jugular vein were clearly distinguishable (figure 2.2). This generally allows an overview of the vessel orientation, wall thickness, plaques, and surrounding structures. The probe was then placed on the common carotid artery and then the probe was turned to the horizontal orientation until widening of the common carotid artery was achieved.

Measurements were made bilaterally (both on the left and the right sides of the neck) from three angles; lateral or direct, anterior and posterior views (figures 2.3 -2.5). For the lateral view, the probe was positioned directly perpendicular to the neck at approximately a 45 ° angle to the shoulder. For the anterior view, the probe was moved to the front of the neck roughly perpendicular to the shoulder and for the posterior view the probe was moved to the side of the neck roughly parallel with the shoulder.

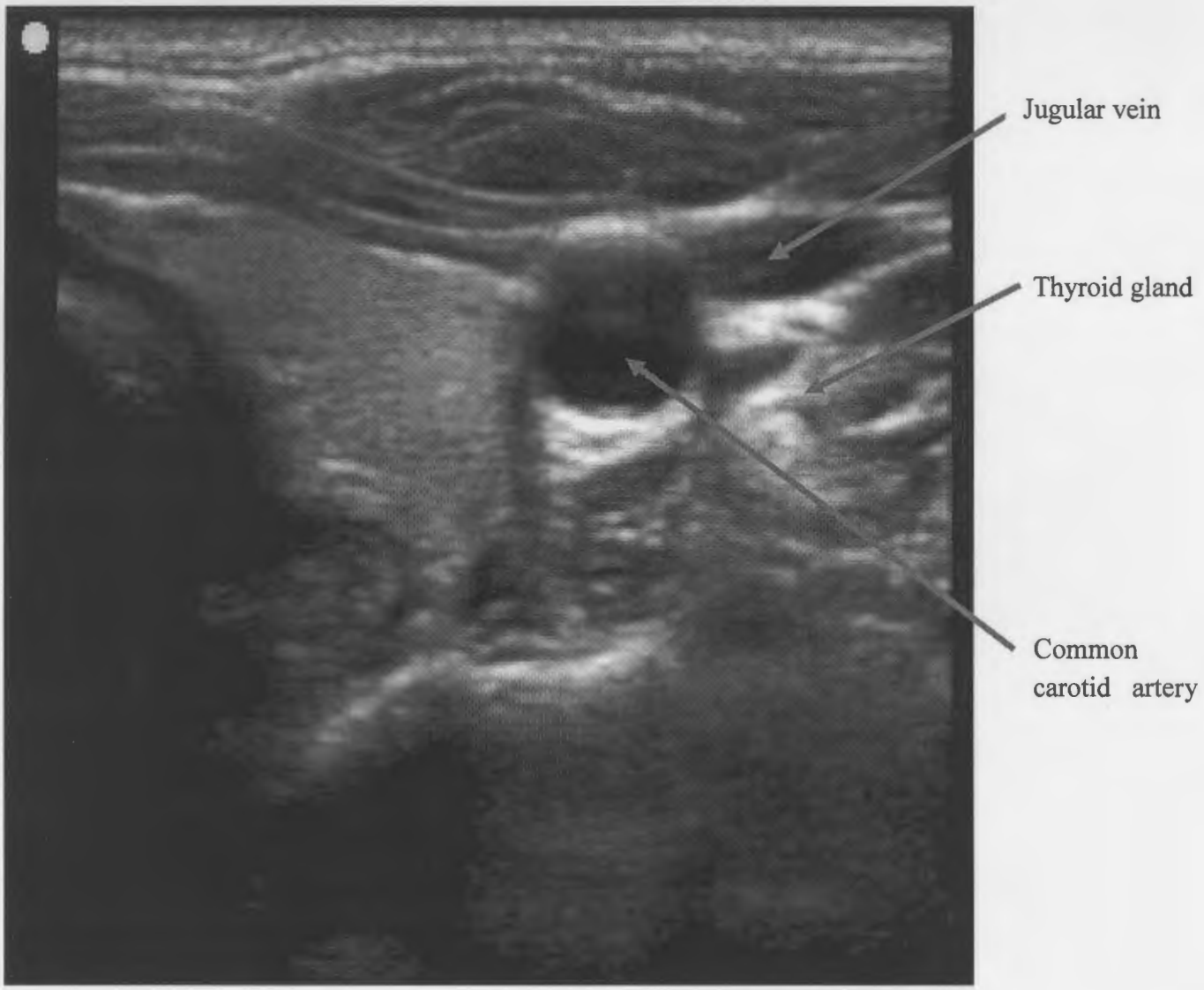

Figure 2.2 A transverse view of the neck showing the common carotid artery, thyroid gland and jugular vein in transverse section.

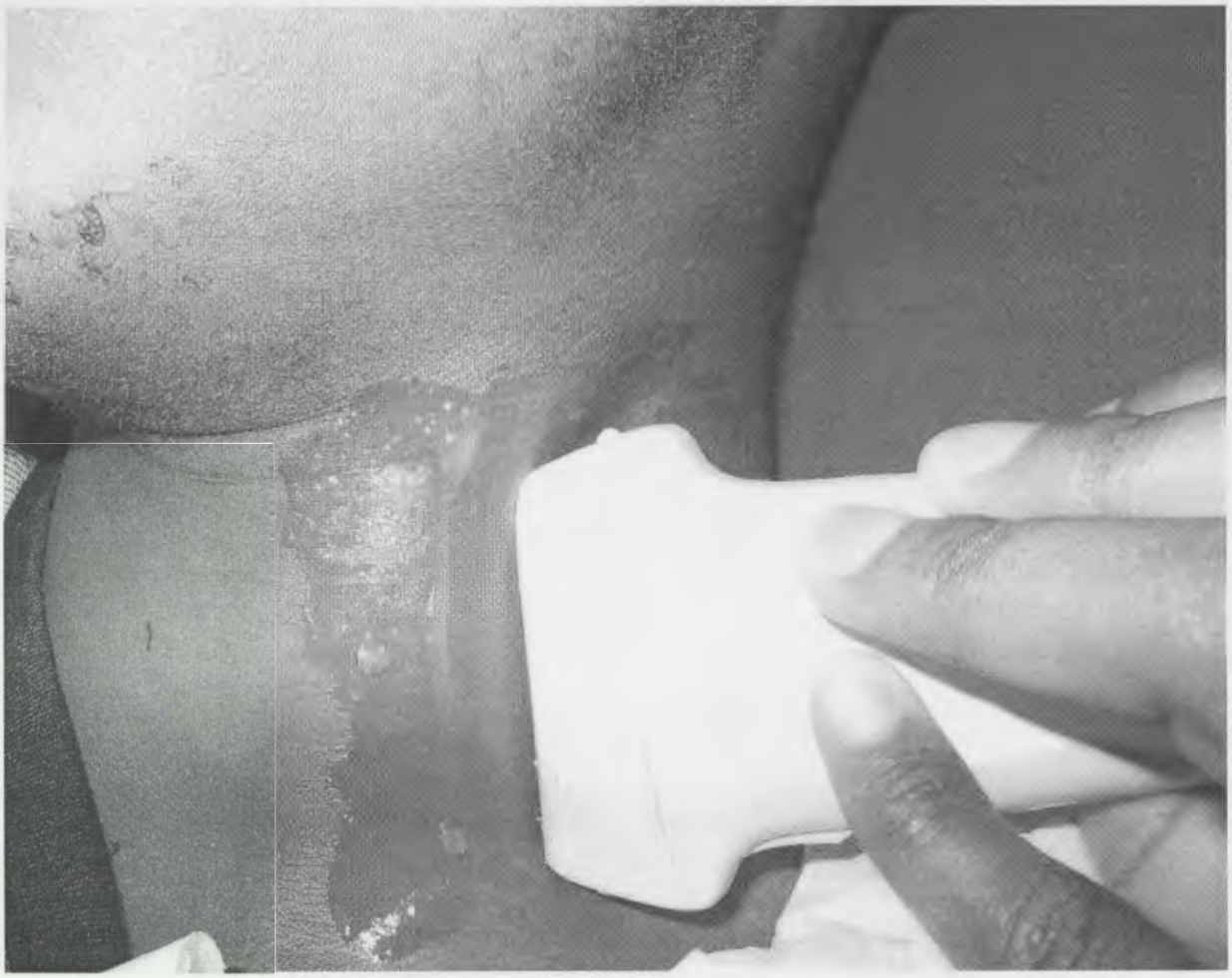

Figure 2.3 A lateral view with the L38 probe placed laterally on the left side of the neck with the participant lying down.

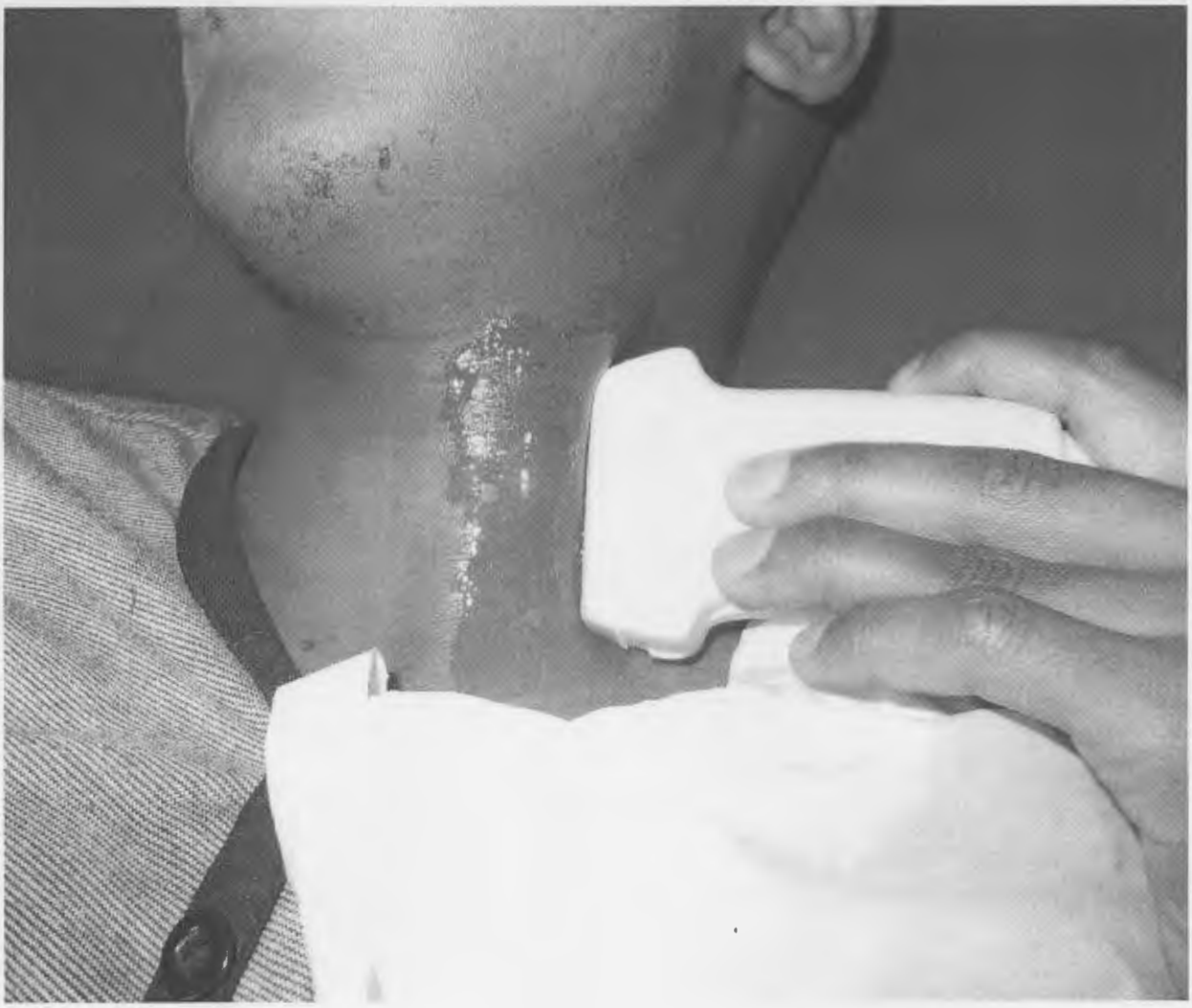

Figure 2.4 A posterior view with the L38 probe placed perpendicular to the neck and parallel with the shoulder on the posterior side of the neck.

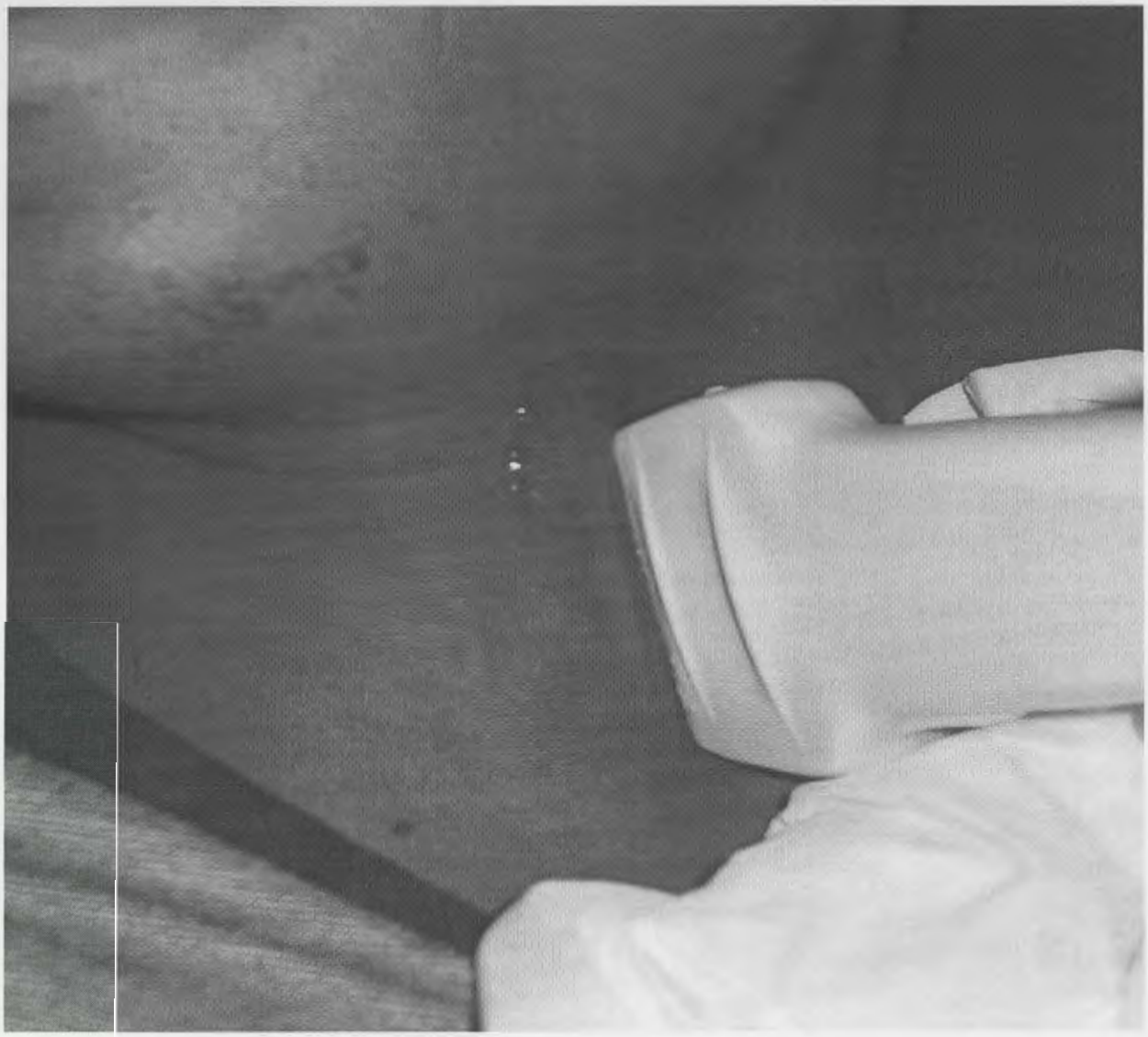

Figure 2.5 An anterior view with the L38 probe placed on the anterior left side of the neck with the participant lying down.

To find the longest, clearest image of a flat segment of the common carotid artery, which was horizontally orientated on the MicroMaxx screen, the probe was manipulated by rotating or keeling and towing (rocking the probe up and down lengthwise). The measure lines (two horizontal yellow lines in figures 2.6 and 2.7) for the measurement of the C-IMT were placed in the distal segment of the common carotid artery 10 mm before the bifurcation or bulb (Bensen *et al.* 1999; Urbina *et al.* 2009) (figure 2.6), as C-IMT increases closer to the bifurcation. Caution was taken that a good clear and non-tortuous image of the carotid artery in the horizontal plane was obtained which included the bulb or bifurcation of the artery and showed the near wall (top), the far wall (bottom) and the lumen (figure 2.6). To ensure reproducibility, approximately 3 to 6 measurements at each angle (anterior, posterior and lateral views) were taken. Reproducibility was assessed from the mean and standard deviation of these measurements.

The computerized edge detecting system (SonoSite MicroMaxx) uses algorithms to analyse ultrasound (doppler) images (one at a time) and calculate IMT value(s) (the IMT is the thickness between the two horizontal yellow lines in figures 2.6 and 2.7). Using the computerized edge detecting system, the width of the intima-media (between the two vertical grey lines in figure 2.7), the mean thickness of the intima-media (between the two yellow lines in figure 2.7), and the maximum intima-media thickness (between the two small blue lines in figure 2.7) were measured. This edge-detection software has become the accepted standard for C-IMT measurement (Bots *et al.* 1997; Cobble & Bale 2010), as the use of this software improves C-IMT reproducibility and reduces observer bias compared with manual techniques. Caution was taken that the measure-lines followed the contour of the intima-media layer precisely. The images were then saved each time a satisfactory image and C-IMT measurement was captured.

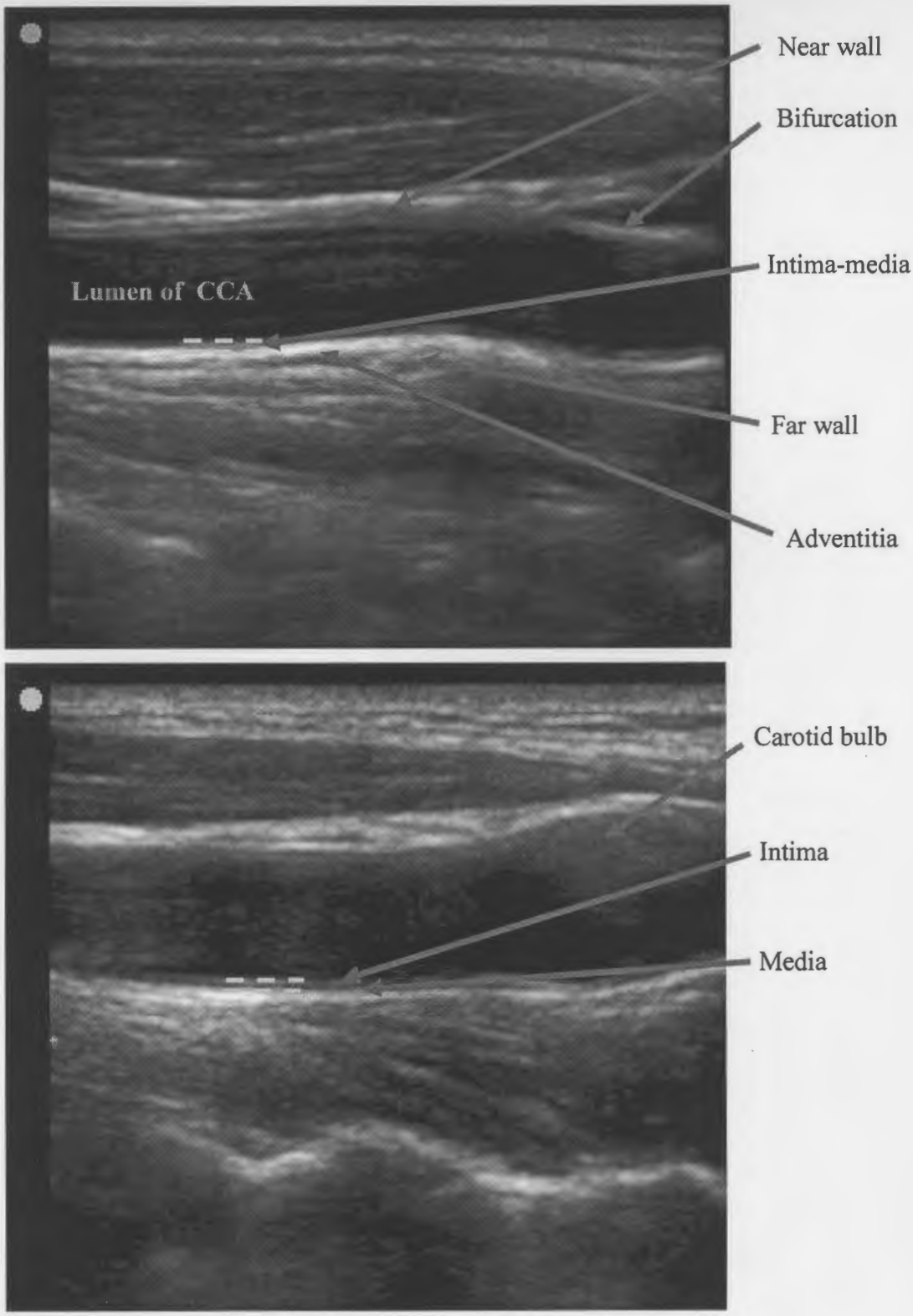

Figure 2.6 Images of the common carotid artery showing the bulb (below) and the bifurcation (above), the lumen, the near and far walls, the intima, media, adventitia and the intima-media (between the dotted yellow lines). CCA, common carotid artery.

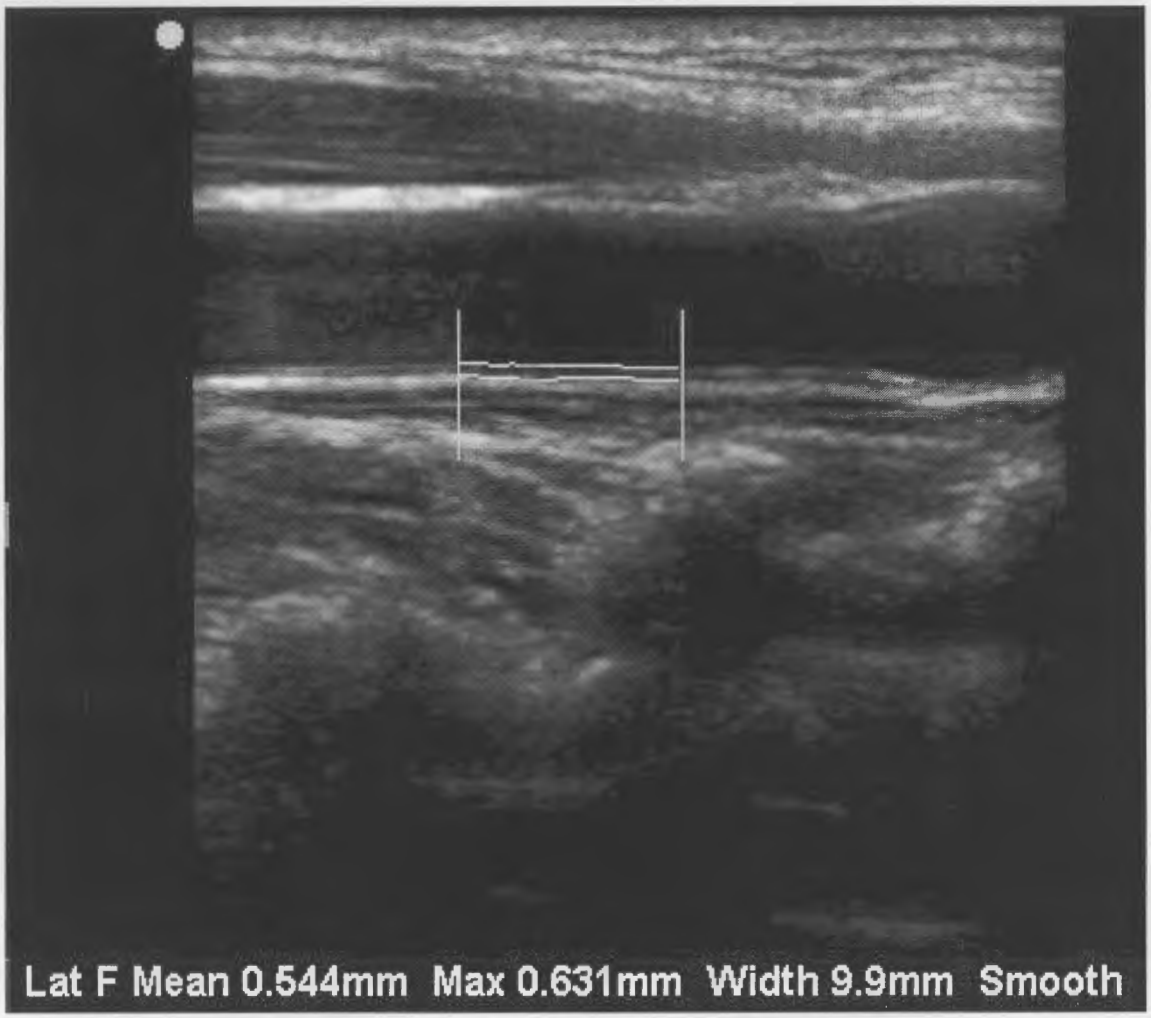

Figure 2.7 A clear B-mode ultrasound image of the measurement of the C-IMT of the far wall as detected using the computerized edge detecting system that uses algorithms to analyze ultrasound (doppler) images and calculate the IMT value. The two vertical grey lines indicate the width of the intima-media layer and the horizontal yellow lines measure the mean thickness of the IM layer. The two small blue lines within the yellow lines indicate the maximum IM layer.

In addition, the saved images were transferred to a personal computer with a SonoCalc. program (Sonosite, Inc.) for the manual examination and confirmation of the C-IMT at a later stage. The mean C-IMT from the lateral (direct) angle, as this is the most accepted measurement (Casella *et al.* 2008), was recorded for each participant and a report generated (figure 2.8). The average of each participant's mean C-IMT was compared to results of large reference populations (table 2.1) and interpreted for cardiovascular disease risk assessment based on percentile analysis.

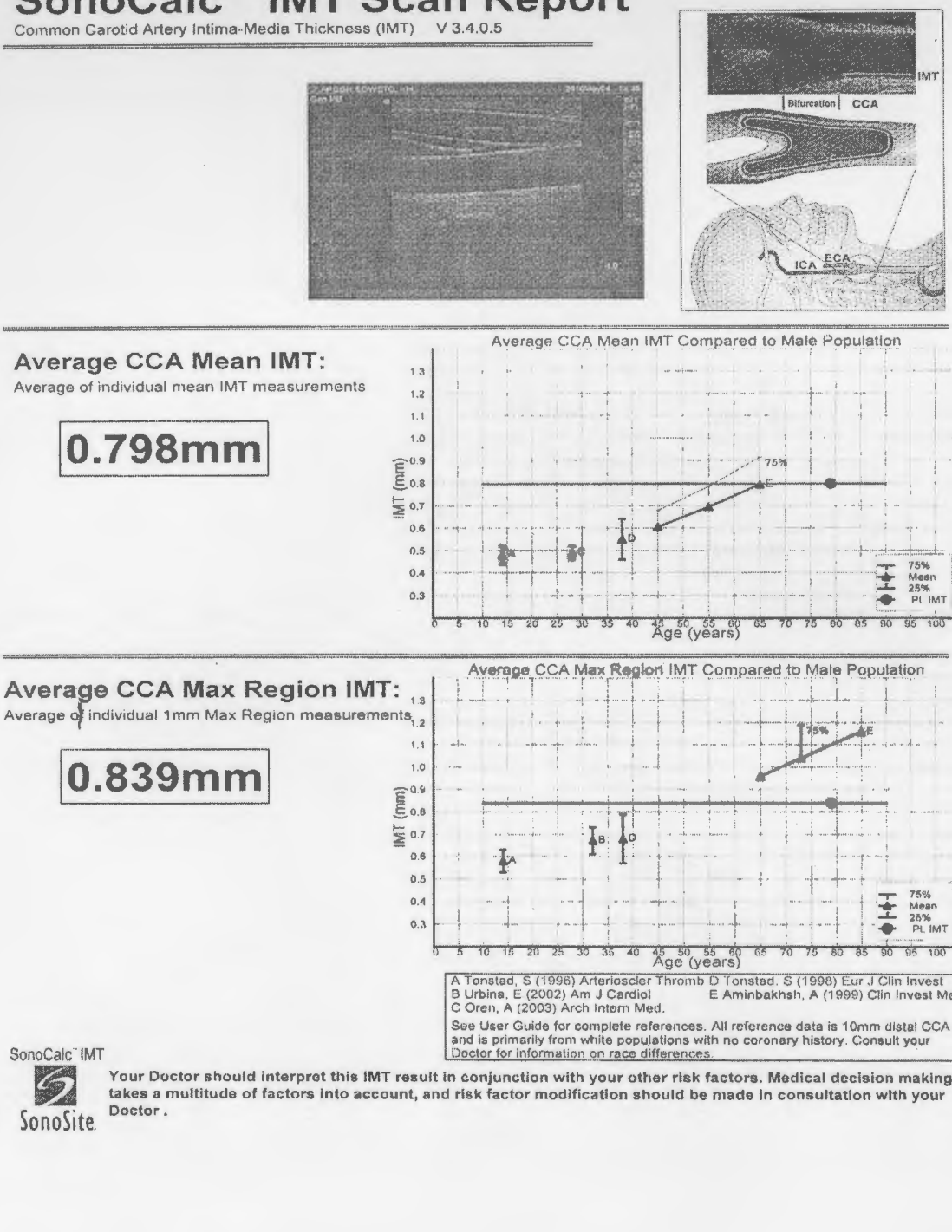

Figure 2.8 A SonoCalc. IMT scan report after manual examination of the C-IMT of a participant from our clinic. The report shows the image of the carotid artery, the mean C-IMT and percentile values of the participant.

Appendix 1 Common carotid artery carotid intima-media thickness values and percentiles from large North American cohort studies

A. Mean far wall common carotid artery carotid intima-media thickness values from the Atherosclerosis Risk in Communities Study[76]

Right

	White male			White female			Black male			Black female		
Age, y/percentile	45	55	65	45	55	65	45	55	65	45	55	65
25th	0.496	0.572	0.648	0.476	0.542	0.608	0.514	0.614	0.714	0.518	0.578	0.638
50th	0.570	0.664	0.758	0.536	0.616	0.696	0.604	0.724	0.844	0.588	0.668	0.748
75th	0.654	0.774	0.894	0.610	0.710	0.810	0.700	0.850	1.000	0.664	0.764	0.864

Left

	White male			White female			Black male			Black female		
Age, y/percentile	45	55	65	45	55	65	45	55	65	45	55	65
25th	0.524	0.588	0.652	0.472	0.540	0.608	0.530	0.610	0.690	0.494	0.558	0.622
50th	0.598	0.684	0.770	0.538	0.622	0.706	0.614	0.714	0.814	0.566	0.646	0.726
75th	0.690	0.806	0.922	0.610	0.710	0.810	0.704	0.840	0.976	0.644	0.748	0.852

B. Maximum far wall common carotid artery carotid intima-media thickness values from the Bogalusa Heart Study[77]

Right

	White male				White female				Black male				Black female			
Age, y/percentile	25	30	35	40	25	30	35	40	25	30	35	40	25	30	35	40
25th	0.611	0.636	0.662	0.687	0.562	0.586	0.611	0.635	0.637	0.675	0.712	0.750	0.616	0.650	0.685	0.719
50th	0.663	0.702	0.740	0.779	0.633	0.654	0.676	0.697	0.719	0.756	0.793	0.830	0.682	0.718	0.754	0.790
75th	0.768	0.807	0.845	0.884	0.717	0.735	0.754	0.772	0.839	0.884	0.929	0.974	750	0.793	0.837	0.880

Left

	White male				White female				Black male				Black female			
Age, y/percentile	25	30	35	40	25	30	35	40	25	30	35	40	25	30	35	40
25th	0.577	0.617	0.658	0.698	0.554	0.586	0.618	0.650	0.640	0.676	0.713	0.749	0.587	0.629	0.670	0.712
50th	0.655	0.707	0.760	0.812	0.621	0.657	0.693	0.729	0.736	0.774	0.812	0.850	0.646	0.691	0.736	0.781
75th	0.763	0.814	0.864	0.915	0.660	0.713	0.766	0.819	0.794	0.844	0.894	0.944	0.714	0.768	0.822	0.876

C. Maximum near and far wall common carotid artery carotid intima-media thickness values from the CHS Study (Alice M. Arnold, PhD, personal communication, December 2006)

	Male					Female				
Age, y/percentile	65-69	70-74	75-79	80-84	85+	65-69	70-74	75-79	80-84	85+
25th	0.94	0.95	1.00	1.03	1.05	0.87	0.89	0.92	0.96	0.99
50th	1.03	1.07	1.10	1.15	1.18	1.96	0.99	1.03	1.05	1.12
75th	1.16	1.21	1.25	1.30	1.32	1.07	1.10	1.16	1.19	1.28

D. Common carotid artery carotid intima-media thickness values from the Multi-Ethnic Study of Atherosclerosis Study (Robyn L. McClelland, PhD, personal communication, January 2007)[6]

Mean far wall-right

	White male				White female				Black male				Black female			
Age, y/percentile	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.52	0.57	0.65	0.72	0.51	0.55	0.65	0.72	0.58	0.61	0.71	0.74	0.55	0.60	0.65	0.71
50th	0.62	0.68	0.77	0.83	0.58	0.65	0.75	0.83	0.67	0.74	0.85	0.85	0.64	0.71	0.76	0.83
75th	0.71	0.81	0.92	0.97	0.67	0.76	0.87	0.93	0.80	0.92	0.99	1.02	0.74	0.81	0.92	0.96

Table 2.1 The reference chart for C-IMT values of various population groups indicating the percentile ranges for ethnicity, sex and age (Stein *et al.* 2008).

Appendix 1 Continued

Age, y/percentile	Chinese male				Chinese female				Hispanic male				Hispanic female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.54	0.56	0.62	0.66	0.55	0.54	0.59	0.67	0.53	0.60	0.65	0.71	0.51	0.57	0.65	0.63
50th	0.64	0.70	0.73	0.79	0.60	0.63	0.71	0.77	0.62	0.67	0.78	0.81	0.58	0.69	0.76	0.78
75th	0.73	0.83	0.92	0.98	0.70	0.77	0.84	0.96	0.73	0.82	0.90	0.92	0.67	0.77	0.87	0.92

Mean far wall–left

Age, y/percentile	Chinese male				Chinese female				Hispanic male				Hispanic female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.55	0.57	0.62	0.69	0.49	0.52	0.58	0.64	0.55	0.61	0.68	0.72	0.51	0.58	0.62	0.68
50th	0.63	0.70	0.72	0.84	0.58	0.63	0.71	0.76	0.64	0.72	0.80	0.86	0.58	0.68	0.72	0.77
75th	0.73	0.84	0.86	0.97	0.67	0.72	0.87	0.94	0.75	0.85	0.98	0.97	0.68	0.79	0.86	0.91

Age, y/percentile	White male				White female				Black male				Black female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.54	0.57	0.67	0.71	0.50	0.55	0.63	0.70	0.56	0.63	0.69	0.72	0.54	0.59	0.63	0.68
50th	0.63	0.69	0.81	0.85	0.58	0.64	0.73	0.80	0.69	0.75	0.82	0.85	0.63	0.67	0.76	0.78
75th	0.78	0.82	0.95	1.00	0.67	0.75	0.85	0.94	0.81	0.92	0.99	1.02	0.73	0.80	0.90	0.91

Maximum far wall–right

Age, y/percentile	White male				White female				Black male				Black female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.61	0.66	0.73	0.83	0.59	0.66	0.77	0.82	0.66	0.72	0.79	0.83	0.63	0.72	0.72	0.79
50th	0.72	0.79	0.89	0.94	0.67	0.74	0.88	0.94	0.77	0.83	0.94	0.96	0.74	0.83	0.87	0.94
75th	0.87	0.94	1.05	1.11	0.79	0.88	1.00	1.07	0.89	1.05	1.11	1.13	0.87	0.94	1.05	1.10

Age, y/percentile	Chinese male				Chinese female				Hispanic male				Hispanic female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.66	0.63	0.66	0.72	0.62	0.61	0.66	0.72	0.61	0.67	0.72	0.78	0.61	0.67	0.72	0.72
50th	0.75	0.79	0.83	0.90	0.72	0.72	0.80	0.88	0.74	0.82	0.88	0.89	0.67	0.77	0.87	0.88
75th	0.86	0.94	1.05	1.07	0.83	0.82	0.94	1.05	0.87	0.95	1.05	1.05	0.78	0.91	1.00	1.03

Maximum far wall–left

Age, y/percentile	White male				White female				Black male				Black female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.64	0.68	0.77	0.77	0.61	0.66	0.72	0.82	0.66	0.72	0.82	0.83	0.62	0.66	0.72	0.77
50th	0.73	0.79	0.90	0.97	0.67	0.77	0.84	0.94	0.79	0.86	0.93	0.95	0.72	0.78	0.84	0.89
75th	0.89	0.94	1.09	1.12	0.78	0.88	1.00	1.11	0.94	1.04	1.11	1.11	0.86	0.94	1.03	1.00

Age, y/percentile	Chinese male				Chinese female				Hispanic male				Hispanic female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.65	0.64	0.72	0.77	0.61	0.61	0.66	0.72	0.62	0.72	0.77	0.77	0.61	0.66	0.72	0.77
50th	0.75	0.79	0.81	0.94	0.72	0.73	0.82	0.83	0.72	0.83	0.94	0.94	0.66	0.77	0.83	0.88
75th	0.88	0.95	1.00	1.06	0.80	0.83	0.96	1.05	0.88	0.97	1.11	1.11	0.78	0.89	0.97	1.02

Y, years. All values are in mm.

Table 2.1 continued, showing reference values for Black males and females (highlighted by red box).

2.2 Data analysis

For database management and statistical analysis, SAS software, version 9.1 (SAS Institute Inc., Cary, NC) was used. Data are shown as mean±SD unless otherwise specified. Differences in mean values between women and men were assessed using large sample Z-test. Bivariate relationships between C-IMT and other variables were assessed using Pearson's correlations. Independent relationships between C-IMT and anthropometric data or lipid profile data were assessed in multivariate models including those factors which were associated with C-IMT on bivariate analysis (age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status and diabetes mellitus), as well as in models which also included those factors associated with C-IMT in the literature (heart rate, current smoking, regular alcohol consumption and gender). Multivariate analysis was performed in all participants as well as in gender specific groups (as female gender was independently associated with C-IMT in both the model with anthropometric data and the model with lipid profile data). Bivariate relationships between C-IMT and the interactions between anthropometric data and lipid profiles were assessed using Pearson's correlations. Independent relationships between C-IMT and the anthropometric data - lipid profile interactive terms were assessed in multivariate models including age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status, diabetes mellitus, heart rate, current smoking, regular alcohol consumption and gender. To determine whether anthropometric data or lipid profiles were associated with C-IMT independent of each other, multivariate regression analyses were performed first without and then with confounders (age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status, diabetes mellitus, heart rate, current smoking, regular alcohol consumption and gender). A p-value of <0.05 was chosen to indicate statistical significance.

Chapter 3

Results

3.0 Results

3.1 Characteristics of the participants

Table 3.1 gives the demographic and clinical characteristics of all participants, as well as men and women separately. More women 278 (65%) than men 152 (35%) participated in the study, and 137 of the women were postmenopausal. In the whole population group, 192 people had hypertension, but only 112 (58% of those who had hypertension) were receiving treatment for hypertension (table 3.1). Although similar proportions of men and women had hypertension, more women than men were treated for hypertension. The number of participants who regularly consumed alcohol or were current smokers was fairly low; however more men than women regularly consumed alcohol or were current smokers (table 3.1). Heart rate was higher in the women than in the men. No other differences were noted between men and women. The C-IMT values were relatively normal with 92% of the values (93% of the values in men and 91% of the values in women) being below the 75th percentile for age and gender (according to the chart in table 2.1). The proportion of all participants with increased C-IMT (>75th percentile for age and gender) was low (8%). More women than men had C-IMT values between the 50th and 75th percentiles for age and gender; whereas more men (36%) than women (12%) had C-IMT values below the 25th percentile for age and gender.

The anthropometric data for all participants, as well as for men and women separately are shown in table 3.2. A high proportion of the sample was either overweight or obese (64.4%). All anthropometric data were increased in women compared to men (table 3.2). Consequently, more women than men were obese. However, similar proportions were overweight.

Table 3.1 Demographic and clinical characteristics of study participants.

	All	Men	Women
Sample number	430	152	278
Age (years)	44.4±18.2	42.5±19.4	45.4±17.4
C-IMT (mm)	0.63±0.14	0.64±0.17	0.63±0.12
C-IMT >25th percentile for age (%)	44.7	40.1	47.1
C-IMT >50th percentile for age (%)	26.7	17.1	32.0*
C-IMT >75th percentile for age (%)	7.9	6.6	8.6
Clinic SBP/DBP (mm Hg)	128±22/83±1	130±22/84±12	126±21/82±13
Heart rate (bpm)	65.5±12.4	62.5±13.7	67.2±11.4*
MAP (mmHg)	99± 16	101±15	98±16
PWV (m.s^{-1})	6.11±2.73	6.15±2.95	6.10±2.59
Hypertension (%)	44.7	44.1	45.0
Treated for hypertension (%)	26.1	18.4	30.2**
DM (%)	9.1	8.6	9.7
Current smokers (%)	15.8	28.9	8.6**
Regular alcohol consumption (%)	22.8	36.2	15.5**
Postmenopausal (%)	-	-	49.3

Values expressed as mean (± standard deviation) or percentages. *p<0.001, **p<0.0001 versus men. C-IMT, carotid intima media thickness; DBP, diastolic blood pressure; DM, diabetes mellitus; MAP, mean arterial pressure; PWV, pulse wave velocity; SBP, systolic blood pressure; C-IMT above 25th, 50th and 75th percentile for age and gender were as per chart in table 2.1.

Table 3.2 Anthropometric characteristics of study participants.

	All	Men	Women
Sample number	430	152	278
BMI ($kg.m^{-2}$)	29.5±8.3	24.8±6.0	32.1±8.3**
Waist circumference (cm)	90.6±18.1	83.7±14.8	94.3±18.6**
Hip circumference (cm)	108.0±15.9	97.6±12.5	113.7±14.7**
Skinfold thickness (cm)	2.01±1.07	1.26±0.7	2.41±0.97**
WHR	0.84±0.11	0.86±0.11	0.83±0.11*
Overweight (%)	23.0	26.3	21.2
Obese (%)	41.4	15.8	55.4**

Values expressed as mean (± standard deviation) or percentages. *$p<0.01$, **$p<0.0001$ versus men. BMI, body mass index; WHR, waist-to-hip ratio.

The lipid data for all participants, as well as for men and women separately are shown in table 3.3. The lipid profiles of the participants were fairly normal with only a third of all participants having raised total or LDL cholesterol concentrations, and only one fifth of all participants having either raised triglycerides or low HDL cholesterol concentrations. Total cholesterol and LDL cholesterol were increased in women compared to men (table 3.3); whereas the HDL cholesterol and triglycerides did not differ between men and women. More women than men had increased total and LDL cholesterol, and decreased HDL cholesterol.

3.2 Association of C-IMT with potential confounders

In bivariate analysis, C-IMT was strongly associated with age (figure 3.1); clinic systolic blood pressure ($r=0.38$, $p<0.0001$); clinic diastolic blood pressure ($r=0.19$, $p<0.0001$); mean arterial pressure ($r=0.29$, $p<0.0001$); pulse wave velocity ($r=0.49$, $p<0.0001$); diabetes mellitus ($r=0.15$, $p=0.003$); glycated hemoglobin ($r=0.23$, $p<0.0001$); treatment for hypertension ($r=0.39$, $p<0.0001$) and postmenopausal status ($r=0.24$, $p<0.0001$). However, C-IMT was not associated with heart rate ($r=0.07$, $p=0.07$); gender ($r=0.04$, $p=0.373$); current smoking ($r=-0.03$, $p=0.548$) or regular alcohol consumption ($r=-0.08$, $p=0.080$). In men, C-IMT was associated with age ($r=0.67$, $p<0.0001$); systolic blood pressure ($r=0.41$, $p<0.0001$); diastolic blood pressure ($r=0.17$, $p=0.04$); mean arterial pressure ($r=0.31$, $p<0.0001$); pulse wave velocity ($r=0.52$, $p<0.0001$); glycated hemoglobin ($r=0.21$, $p=0.01$) and treatment for hypertension ($r=0.41$, $p<0.0001$); but was not associated with heart rate ($r=0.09$, $p=0.25$); current smoking ($r=-0.05$, $p=0.526$); regular alcohol consumption ($r=-0.09$, $p=0.288$) or diabetes mellitus ($r=0.10$, $p=0.233$). In women, C-IMT was similarly associated with age ($r=0.67$, $p<0.0001$); systolic blood pressure ($r=0.37$, $p<0.0001$); diastolic blood pressure ($r=0.20$, $p<0.001$); mean arterial pressure ($r=0.28$, $p<0.0001$); pulse wave velocity ($r=0.47$,

Table 3.3 Lipid profiles of study participants.

	All	Men	Women
Sample number	430	152	278
Total cholesterol ($mmol.l^{-1}$)	4.60±1.02	4.35±0.95	4.73±1.04**
HDL cholesterol ($mmol.l^{-1}$)	1.41±0.45	1.39±0.55	1.43±0.39
LDL cholesterol ($mmol.l^{-1}$)	2.65±0.90	2.42±0.81	2.77±0.92***
Triglycerides ($mmol.l^{-1}$)	1.16±0.66	1.19±0.67	1.15±0.65
Total : HDL cholesterol ratio	3.49±1.17	3.44±1.17	3.52±1.17
Increased total cholesterol (%)[a]	36.0	26.3	41.4*
Decreased HDL cholesterol (%)[a]	21.9	14.5	25.9*
Increased LDL cholesterol (%)[a]	34.0	23.7	39.6**
Increased Triglycerides (%)[a]	17.9	20.4	16.5

Values expressed as mean (± standard deviation) or percentages. *$p<0.01$, **$p<0.001$, ***$p<0.0001$ versus men. HDL, high density lipoprotein; LDL, low density lipoprotein. [a], As per European guidelines on cardiovascular disease prevention in clinical practice (De Backer *et al.* 2003), total cholesterol≥5 $mmol.l^{-1}$, LDL cholesterol≥3 $mmol.l^{-1}$ and triglycerides≥1.7 $mmol.l^{-1}$ were considered as increased; and HDL cholesterol<1.0 $mmol.l^{-1}$ for men and <1.2 $mmol.l^{-1}$ for women were considered as decreased.

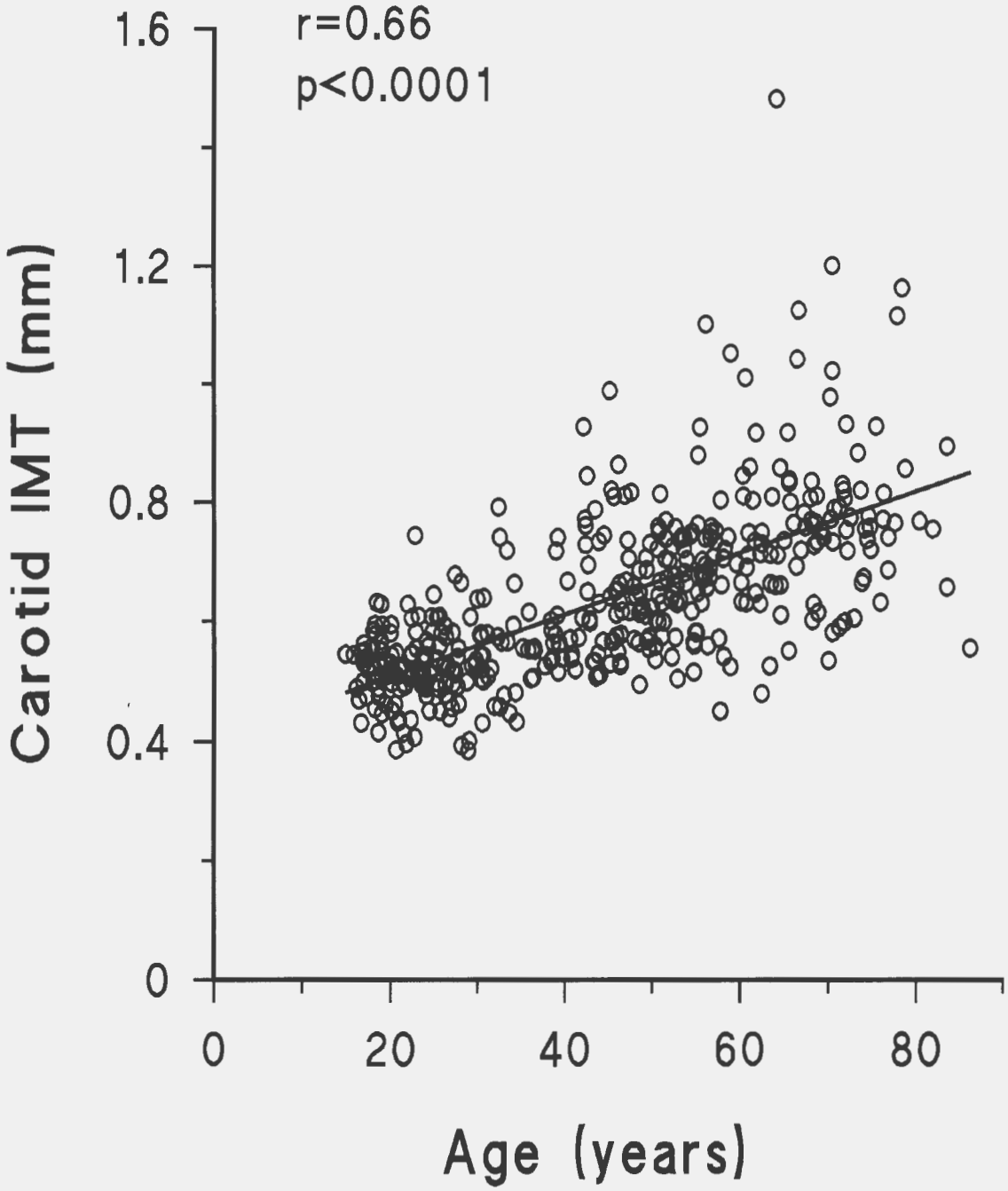

Figure 3.1 Bivariate relationship between C-IMT and age in all participants.

p<0.0001); glycated hemoglobin (r=0.26, p<0.0001); diabetes mellitus (r=0.19, p=0.002); treatment for hypertension (r=0.40, p<0.0001) and postmenopausal status (r=0.58, p<0.0001); but was not associated with heart rate (r=0.10, p=0.10); current smoking (r=-0.03, p=0.612) or regular alcohol consumption (r=-0.11, p=0.070).

3.3 Association of C-IMT with Anthropometric Data

In bivariate analysis, C-IMT was strongly associated with BMI (figure 3.2); waist circumference (figure 3.3); hip circumference (figure 3.4); skinfold thickness (figure 3.5) and waist-to-hip ratio (figure 3.6). The relationships between anthropometric data and C-IMT in men (BMI: r=0.20, p=0.013; waist circumference: r=0.26, p=0.001; hip circumference: r=0.14, p=0.08; skinfold thickness: r=0.26, p=0.002; waist-to-hip ratio: r=0.21, p=0.009) were similar to those in women (BMI: r=0.35, p<0.0001; waist circumference: r=0.38, p<0.0001; hip circumference: r=0.25, p<0.0001; skinfold thickness: r=0.24, p<0.0001; waist-to-hip ratio: r=0.34, p<0.0001).

3.4 Association of C-IMT with Lipid Profile

In bivariate analysis, C-IMT was strongly associated with total cholesterol concentration (figure 3.7); LDL cholesterol concentration (figure 3.8); triglyceride concentration (figure 3.9) and the total-to-HDL cholesterol ratio (figure 3.10); but was not associated with HDL cholesterol concentration (figure 3.11). The relationships between C-IMT and cholesterol concentrations were similar in men (total cholesterol: r=0.24, p=0.003; LDL cholesterol: r=0.22, p=0.006; triglycerides: r=0.25, p=0.002; total-to-HDL cholesterol ratio: r=0.28, p=0.0005) and women (total cholesterol: r=0.28, p<0.0001; LDL cholesterol: r=0.28,

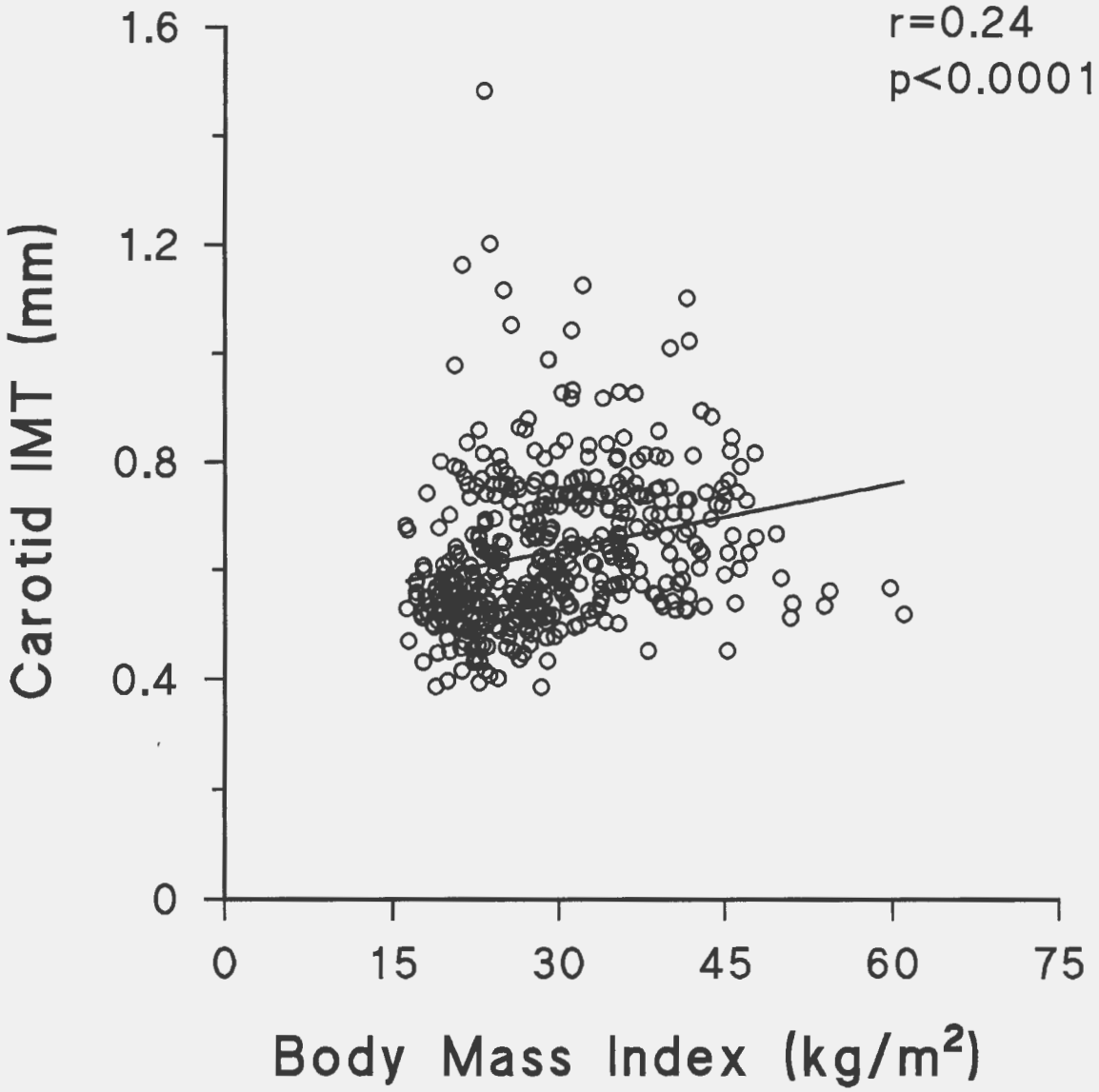

Figure 3.2 Bivariate relationship between C-IMT and BMI in all participants.

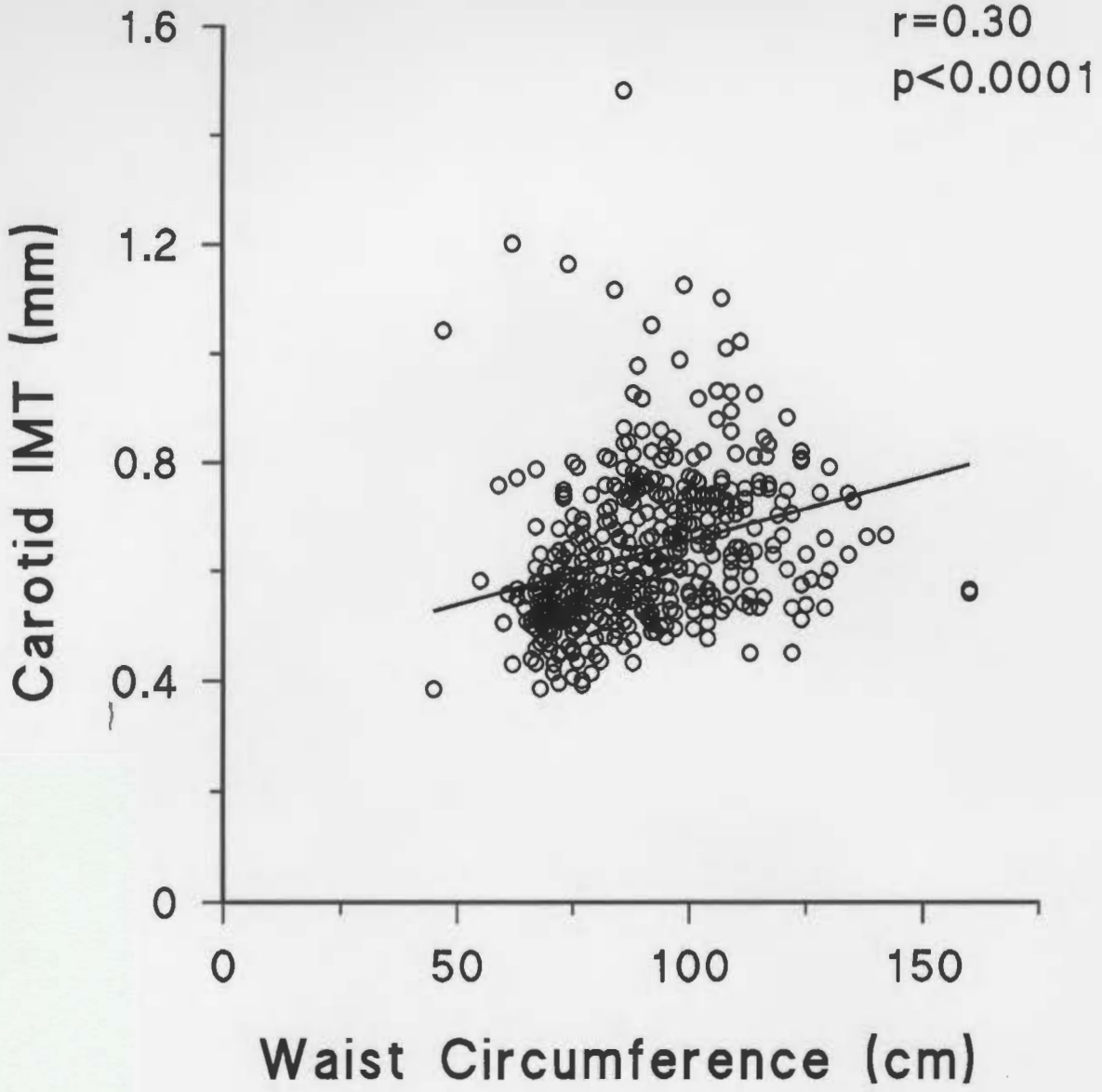

Figure 3.3 Bivariate relationship between C-IMT and waist circumference in all participants.

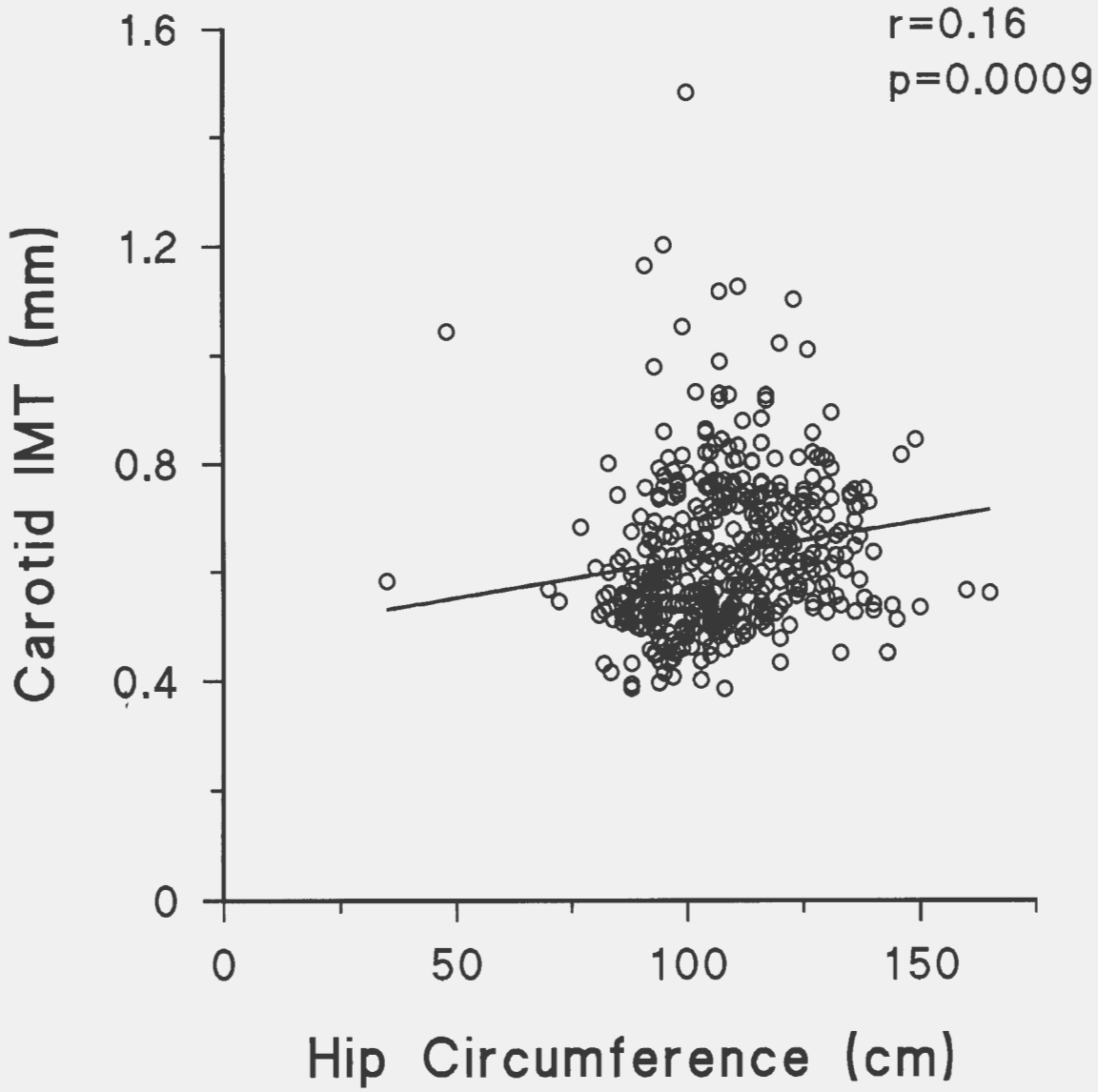

Figure 3.4 Bivariate relationship between C-IMT and hip circumference in all participants.

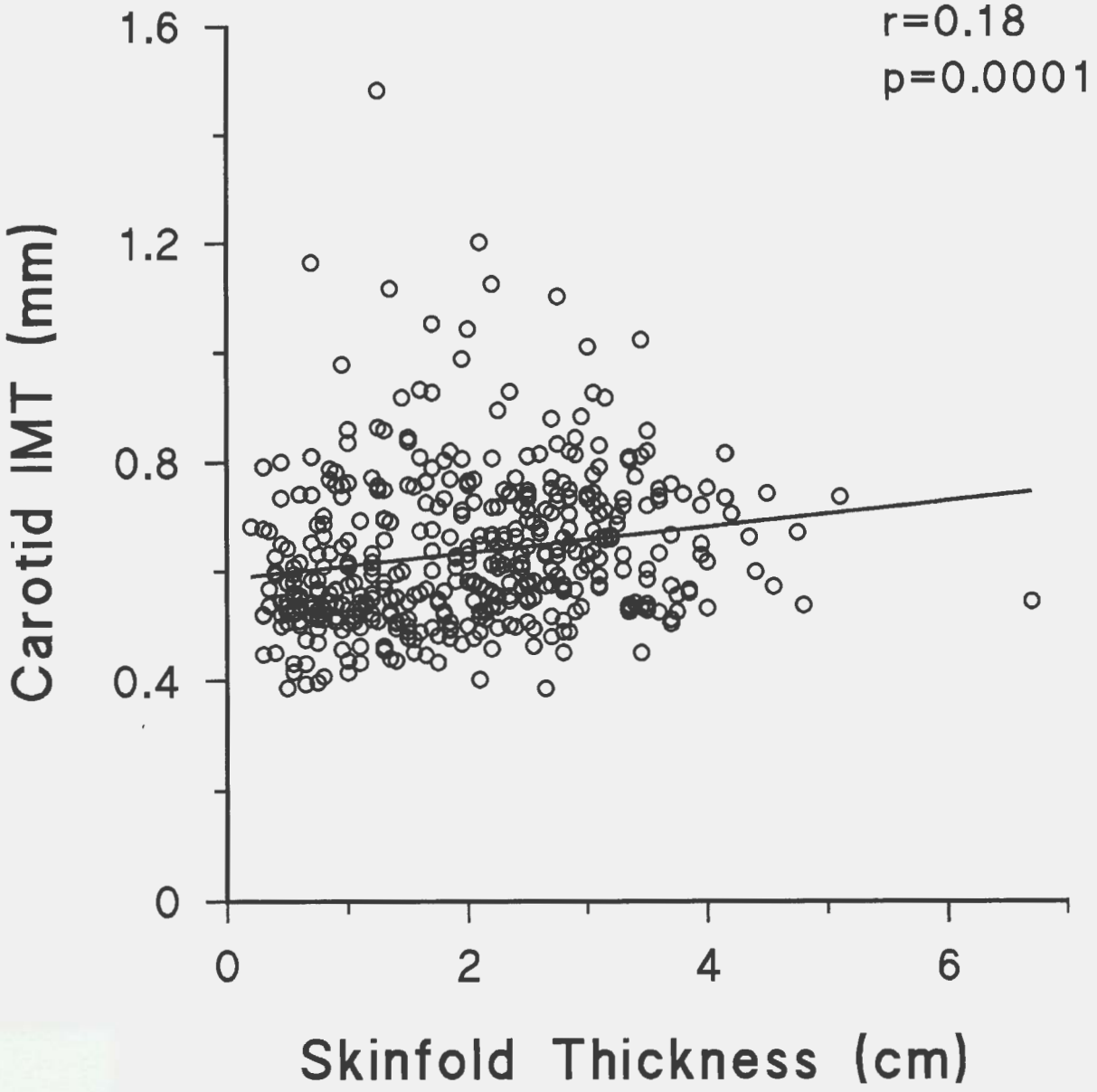

Figure 3.5 Bivariate relationship between C-IMT and skinfold thickness in all participants.

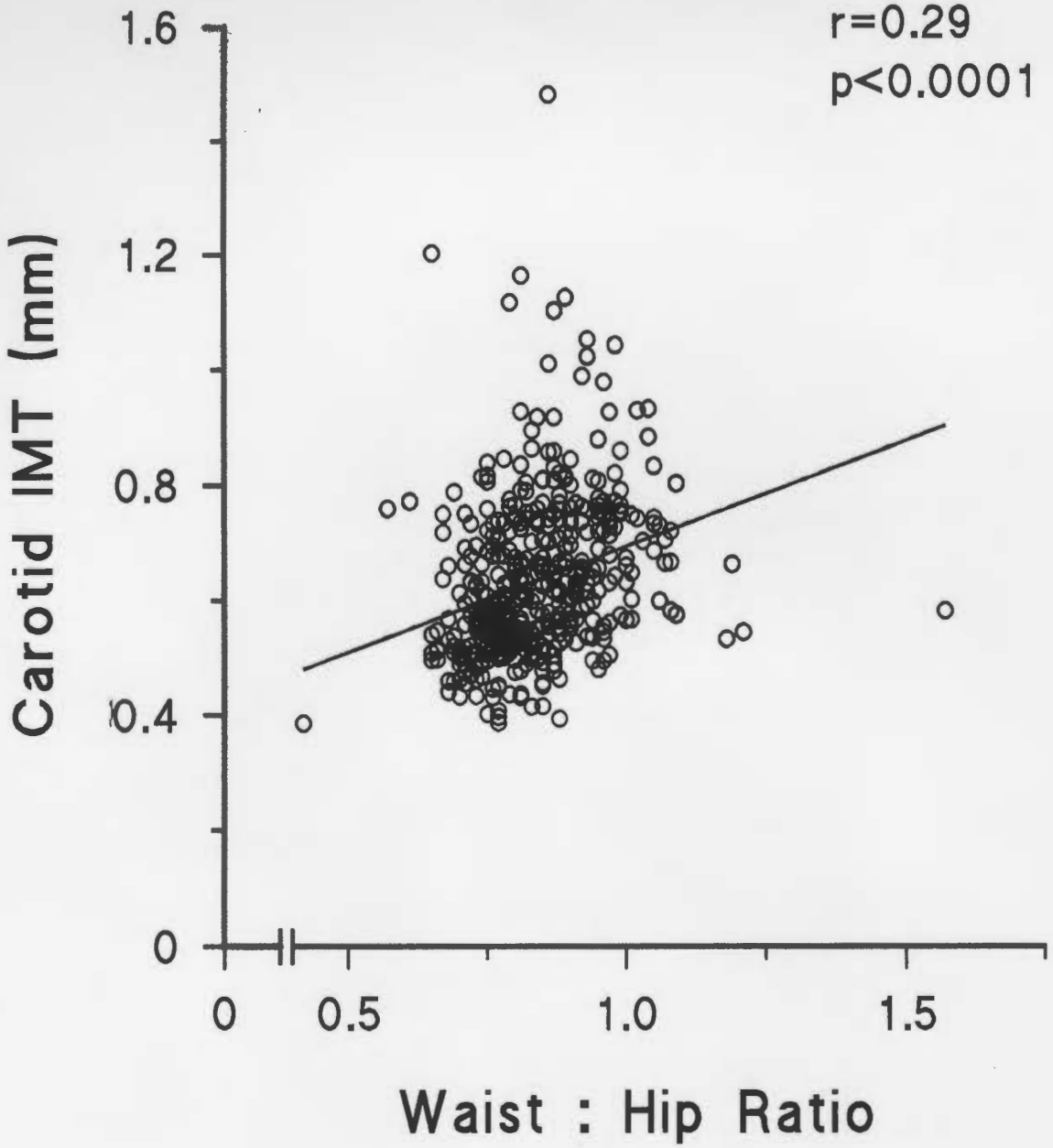

Figure 3.6 Bivariate relationship between C-IMT and waist-to-hip ratio in all participants.

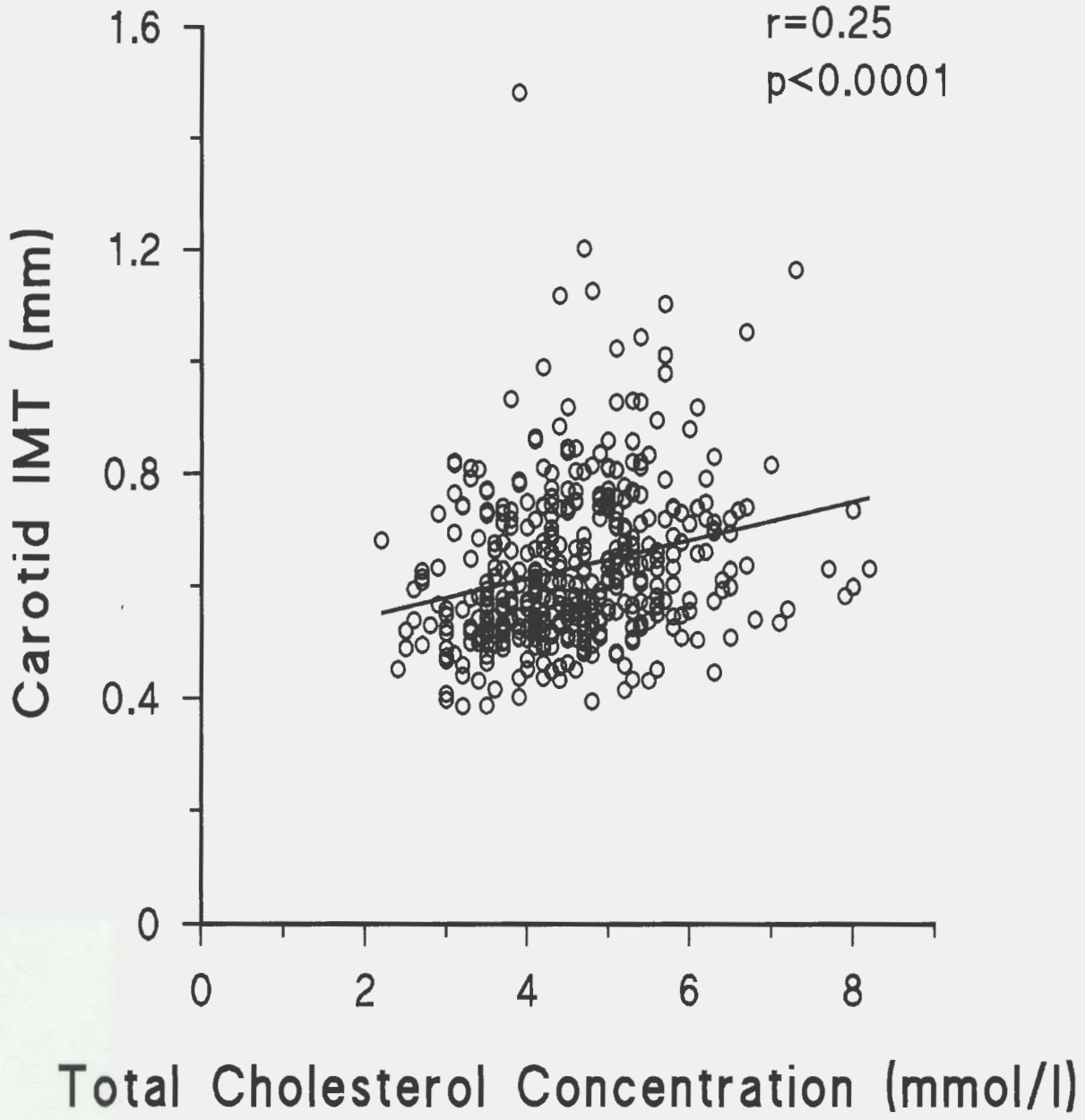

Figure 3.7 Bivariate relationship between C-IMT and total cholesterol concentration in all participants.

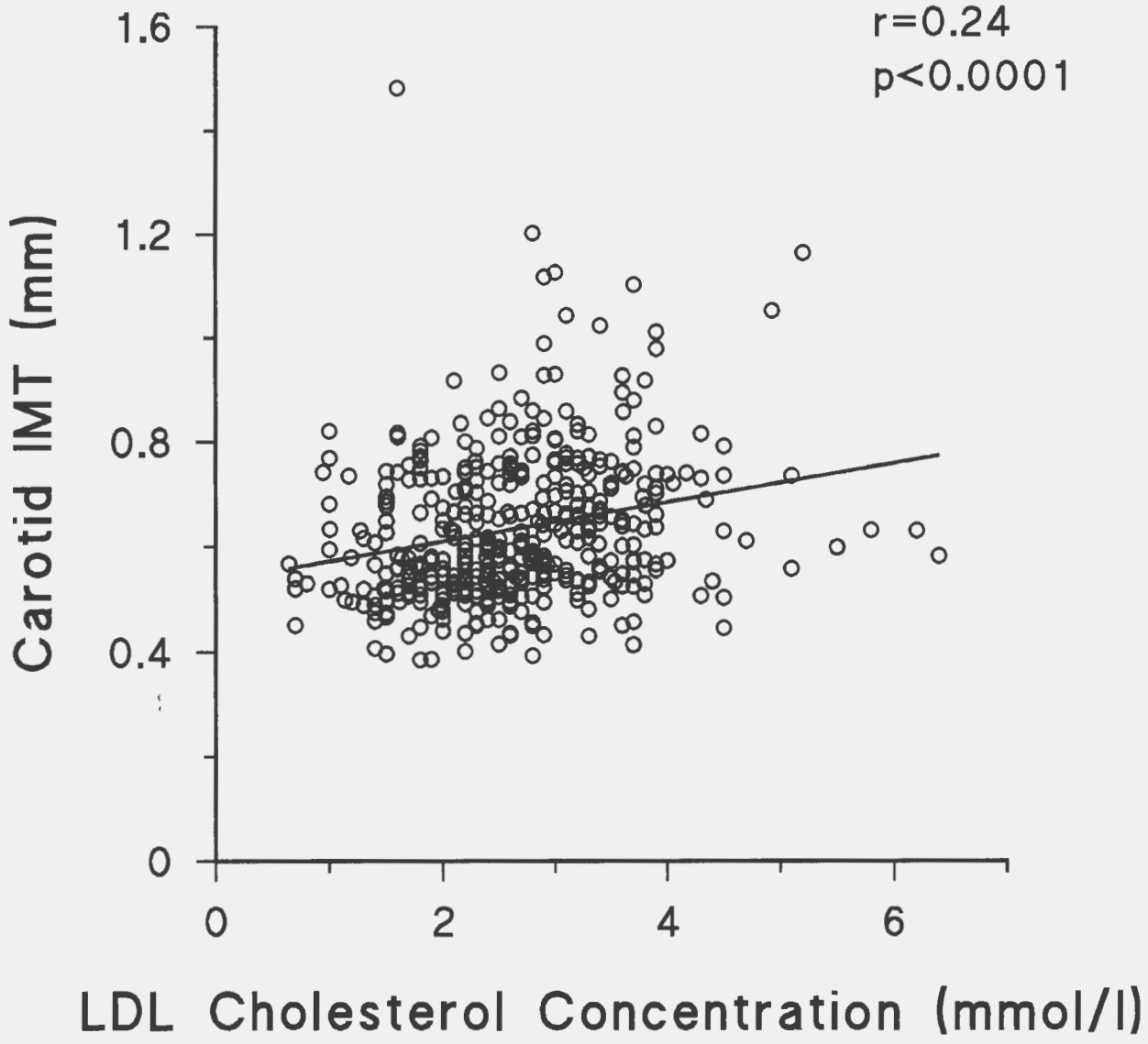

Figure 3.8 Bivariate relationship between C-IMT and LDL cholesterol concentration in all participants.

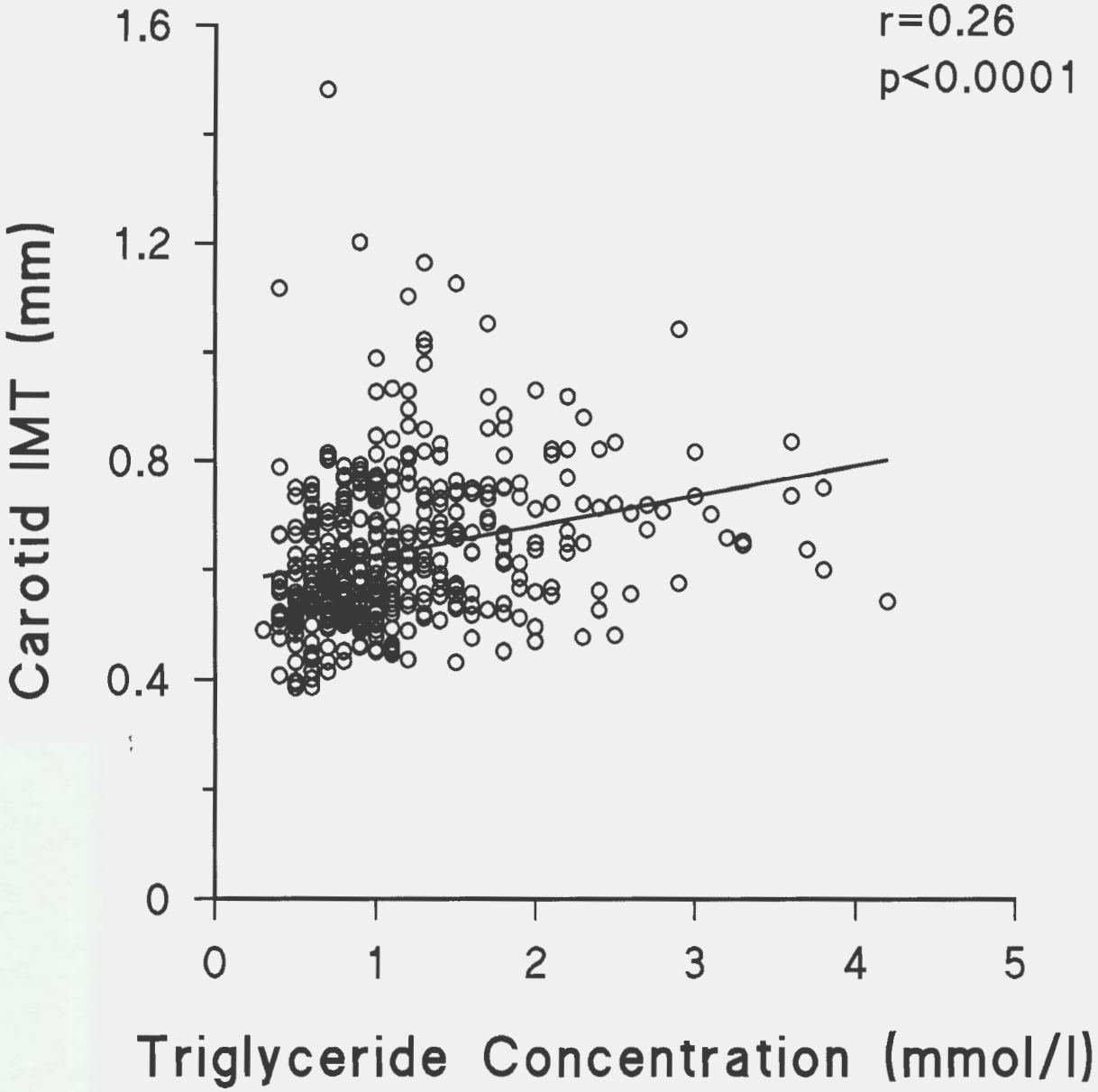

Figure 3.9 Bivariate relationship between C-IMT and triglyceride concentration in all participants.

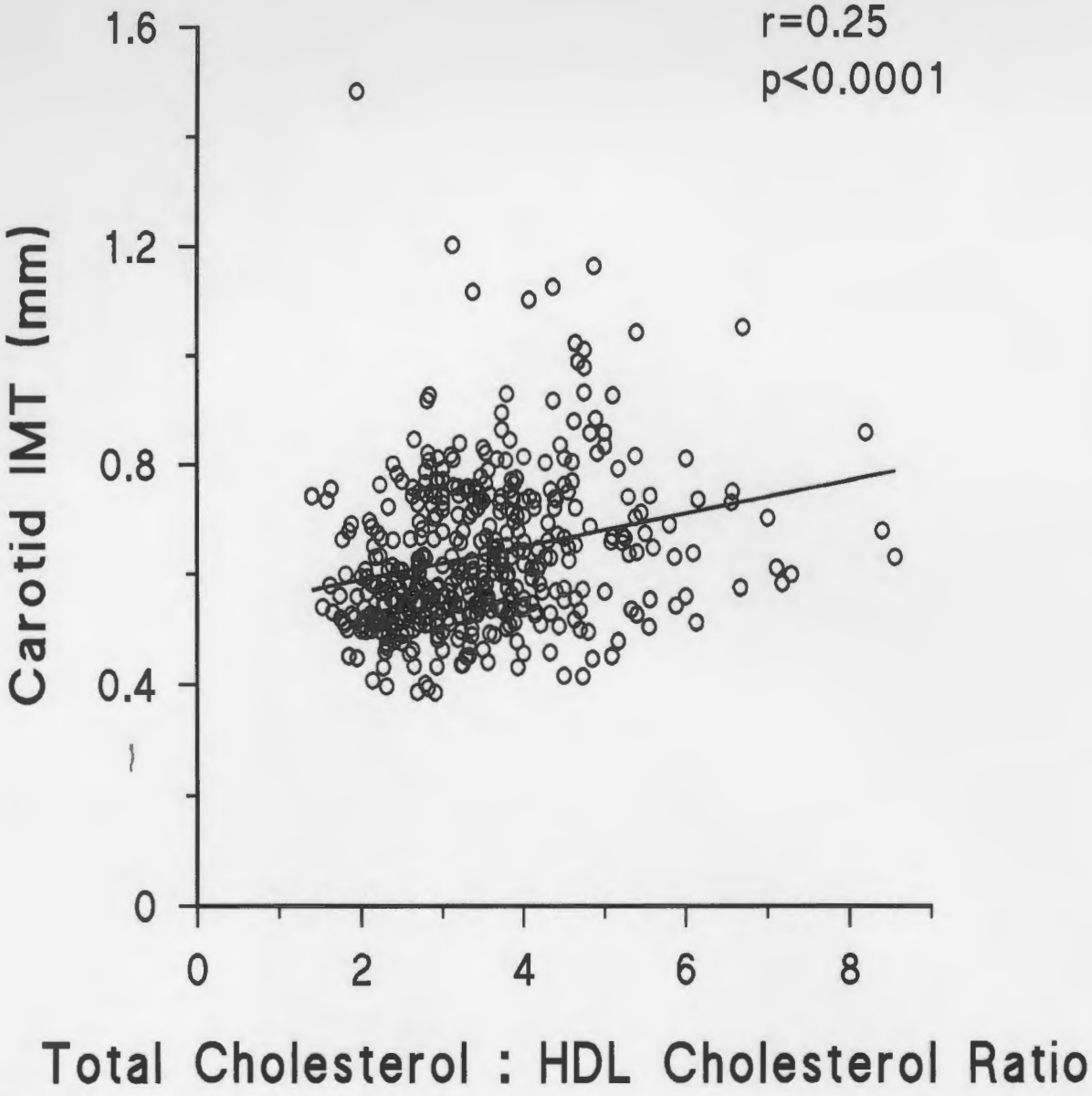

Figure 3.10 Bivariate relationship between C-IMT and total-to-HDL cholesterol ratio in all participants.

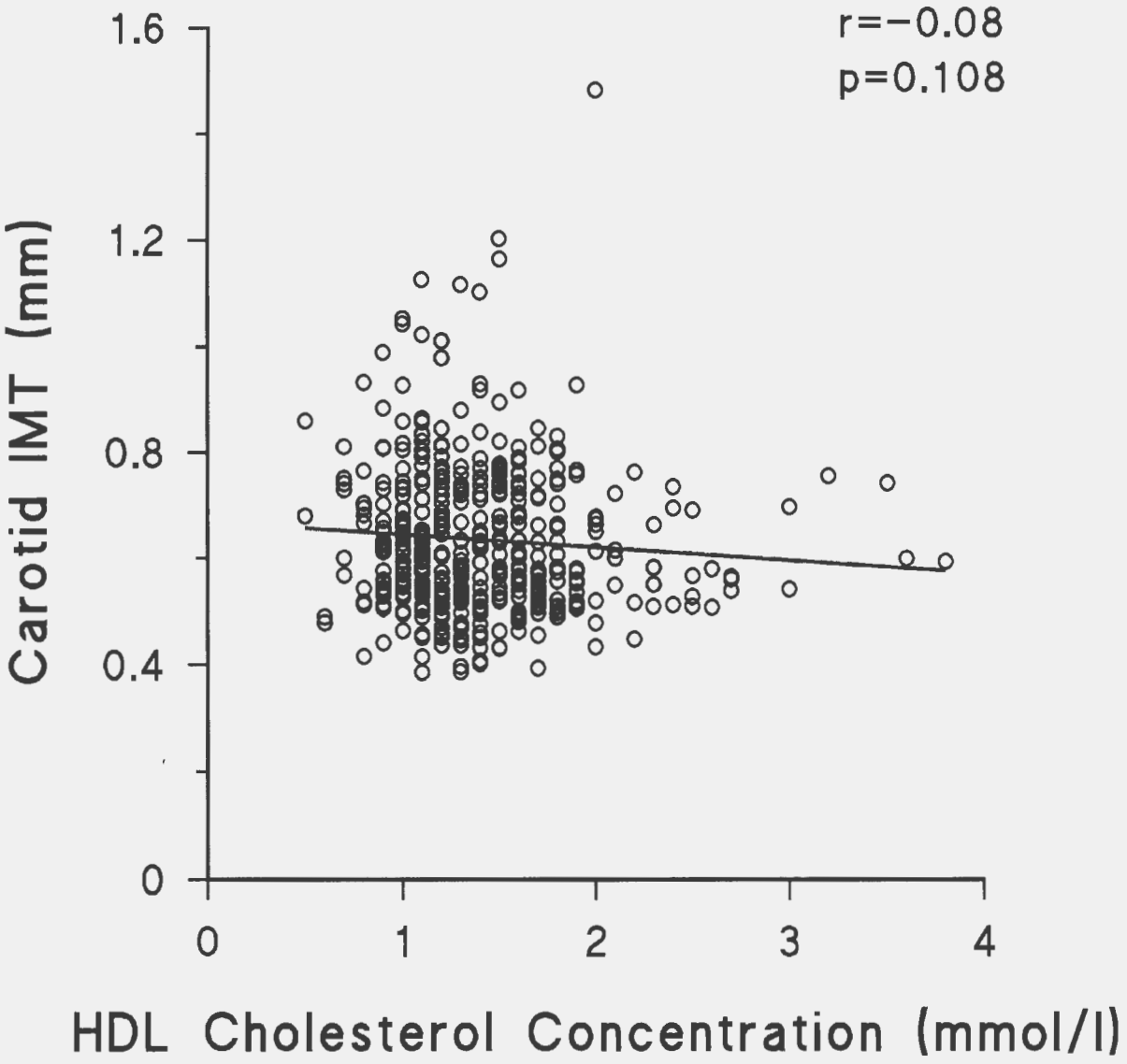

Figure 3.11 Bivariate relationship between C-IMT and HDL cholesterol concentration in all participants.

$p<0.0001$; triglycerides: $r=0.26$, $p<0.0001$; total-to-HDL cholesterol ratio: $r=0.24$, $p<0.0001$). As with the whole group, C-IMT was not associated with HDL cholesterol in either men ($r=-0.05$, $p=0.55$) or women ($r=-0.10$, $p=0.083$).

3.5 Multivariate Regression Model with Anthropometric Data

In multivariate models which included adjustments for the potential confounding factors associated with C-IMT on bivariate analysis (age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status and diabetes mellitus), none of the obesity indices were independently associated with C-IMT (table 3.4). Age was strongly and independently associated with C-IMT in all models ($p<0.0001$) and postmenopausal status was independently associated with C-IMT only in the model with waist-to-hip ratio ($p=0.037$). In addition, in multivariate models which also included adjustments for other potential confounding factors associated with C-IMT in the literature (heart rate, current smoking, regular alcohol consumption and gender in addition to age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status and diabetes mellitus), none of the obesity indices were independently associated with C-IMT (figure 3.12 and table 3.5). Similarly, when men and women were analysed separately, none of the obesity indices were associated with C-IMT independent of age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status (women only), diabetes mellitus, heart rate, current smoking and regular alcohol consumption (figure 3.13, table 3.6 and table 3.7). It is possible that the reason for a negative relationship between WHR and C-IMT in men only may reflect poor nutrition or a catabolic state in men as their mean BMI was 24.8 $kg.m^{-2}$ with 11 (7%) men being below normal range (18.5-24.9 $kg.m^{-2}$. Age was the strongest determinant of C-IMT in all participants (table 3.5), in men (table 3.6) and in women (table 3.7). In all participants, gender was marginally associated with C-IMT (table 3.5).

Table 3.4 Multivariate relationships between C-IMT and anthropometric data in all study participants (n=430).

	Partial r	95% confidence interval	p value
BMI	-0.011	-0.107 to 0.086	=0.826
Waist circumference	-0.031	-0.127 to 0.066	=0.528
Hip circumference	-0.018	-0.114 to 0.078	=0.711
Skinfold thickness	0.013	-0.084 to 0.109	=0.793
Waist-to-hip ratio	-0.035	-0.131 to 0.061	=0.472

Models included age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status and diabetes mellitus in addition to those indicated. Each of the obesity indices were analysed in separate models.

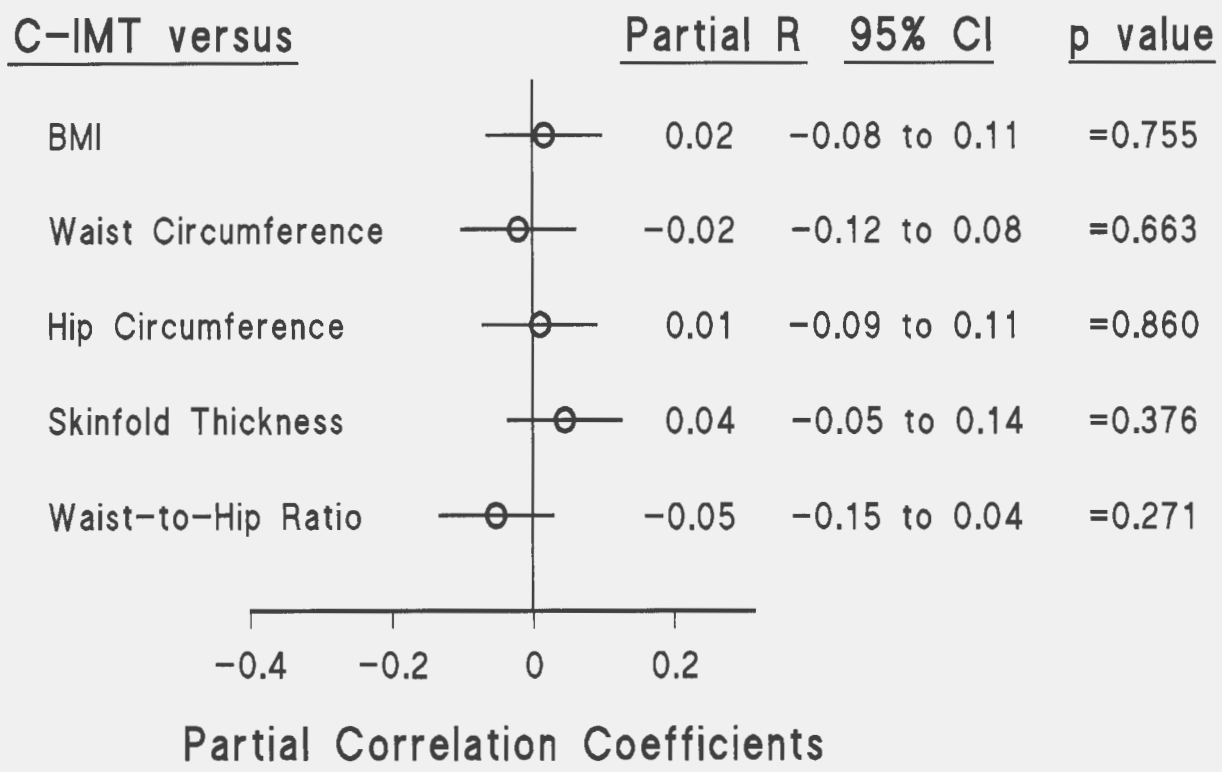

Figure 3.12 Multivariate relationships between C-IMT and anthropometric data in all participants. Age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status, sex, diabetes mellitus, heart rate, current smoking and regular alcohol consumption were included in the models.

Table 3.5 Multivariate relationships between C-IMT and anthropometric data in all study participants (n=430).

	Standardised β-coefficient ± SEM	p value
	Model with BMI	
BMI	0.014 ± 0.045	=0.755
Age	0.657 ± 0.056	<0.0001
Female Sex	0.197 ± 0.093	=0.0348
	Model with Waist circumference	
Waist circumference	-0.014 ± 0.020	=0.663
Age	0.664 ± 0.057	<0.0001
Female Sex	0.183 ± 0.091	=0.0449
	Model with Hip circumference	
Hip circumference	0.008 ± 0.045	=0.860
Age	0.657 ± 0.055	<0.0001
Female Sex	0.194 ± 0.093	=0.0376
	Model with Skinfold thickness	
Skinfold thickness	0.041 ± 0.047	=0.376
Age	0.654 ± 0.055	<0.0001
Female Sex	0.215 ± 0.094	=0.0227
	Model with waist-to-hip ratio	
Waist-to-hip ratio	-0.048 ± 0.044	=0.271
Age	0.675 ± 0.057	<0.0001
Female Sex	0.197 ± 0.090	=0.0288

Models included clinic systolic blood pressure, treatment for hypertension, postmenopausal status, diabetes mellitus, heart rate, current smoking and regular alcohol consumption in addition to those indicated. Each of the obesity indices were analysed in separate models.

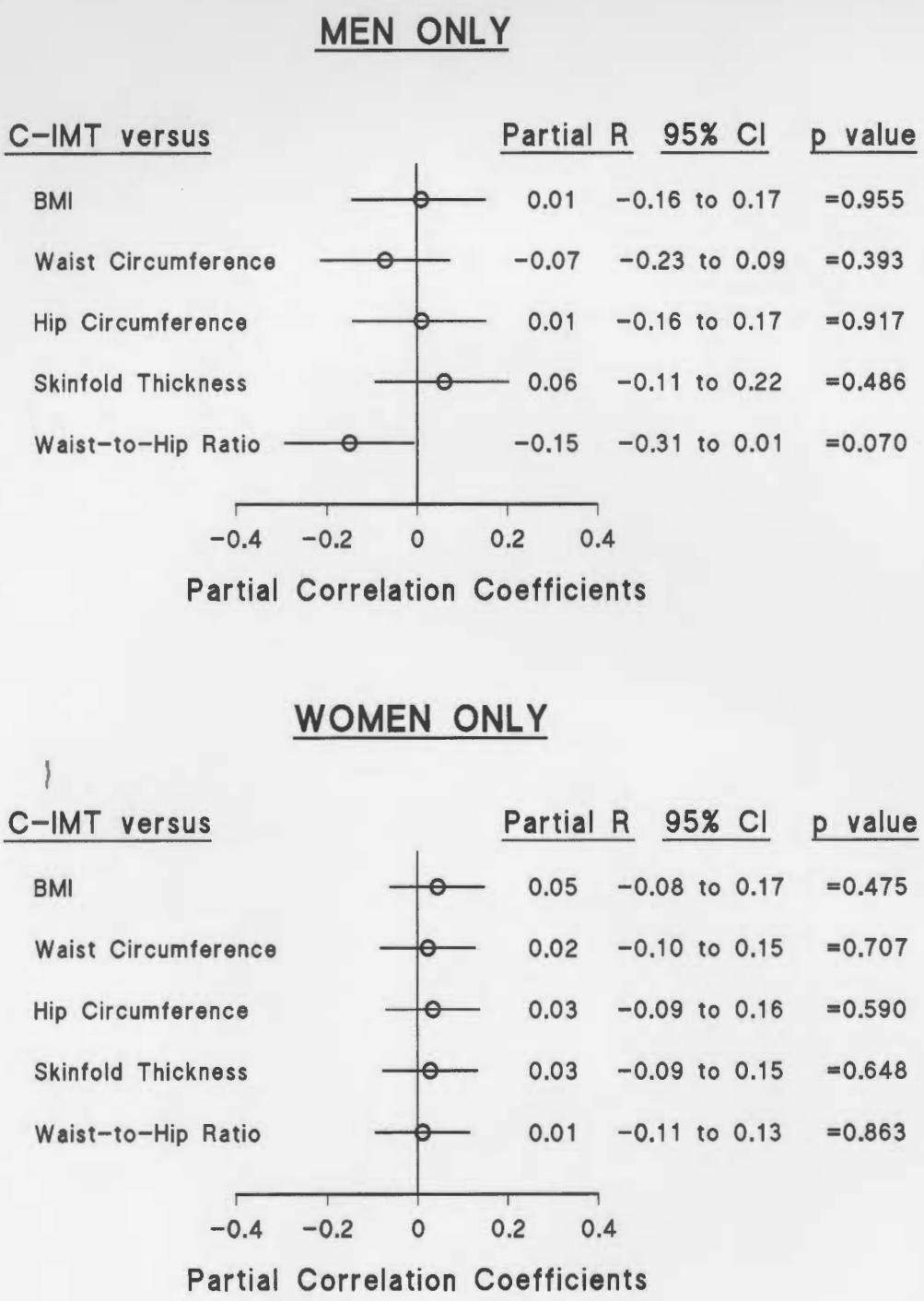

Figure 3.13 Multivariate relationships between C-IMT and anthropometric data in men and women separately. Age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status (women only), diabetes mellitus, heart rate, current smoking and regular alcohol consumption were included in the models.

Table 3.6 Multivariate relationships between C-IMT and anthropometric data in male study participants (n=152).

	Standardised β-coefficient ± SEM	p value
	Model with BMI	
BMI	0.004 ± 0.069	=0.955
Age	0.628 ± 0.086	<0.0001
	Model with Waist circumference	
Waist circumference	-0.065 ± 0.076	=0.393
Age	0.660 ± 0.093	<0.0001
	Model with Hip circumference	
Hip circumference	0.007 ± 0.067	=0.917
Age	0.627 ± 0.087	<0.0001
	Model with Skinfold thickness	
Skinfold thickness	0.049 ± 0.071	=0.486
Age	0.613 ± 0.088	<0.0001
	Model with waist-to-hip ratio	
Waist-to-hip ratio	-0.133 ± 0.073	=0.070
Age	0.676 ± 0.088	<0.0001

Models included clinic systolic blood pressure, treatment for hypertension, diabetes mellitus, heart rate, current smoking and regular alcohol consumption in addition to those indicated. Each of the obesity indices were analysed in separate models.

Table 3.7 Multivariate relationships between C-IMT and anthropometric data in female study participants (n=278).

	Standardised β-coefficient ± SEM	p value
	Model with BMI	
BMI	0.038 ± 0.053	=0.475
Age	0.557 ± 0.087	<0.0001
	Model with Waist circumference	
Waist circumference	0.021 ± 0.055	=0.707
Age	0.559 ± 0.088	<0.0001
	Model with Hip circumference	
Hip circumference	0.027 ± 0.050	=0.590
Age	0.563 ± 0.086	<0.0001
	Model with Skinfold thickness	
Skinfold thickness	0.023 ± 0.050	=0.648
Age	0.566 ± 0.086	<0.0001
	Model with waist-to-hip ratio	
Waist-to-hip ratio	0.010 ± 0.055	=0.863
Age	0.562 ± 0.089	<0.0001

Models included clinic systolic blood pressure, treatment for hypertension, diabetes mellitus, postmenopausal status, heart rate, current smoking and regular alcohol consumption in addition to those indicated. Each of the obesity indices were analysed in separate models.

3.6 Multivariate regression model with lipid profile

In multivariate models which included adjustments for the demographic factors associated with C-IMT on bivariate analysis (age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status and diabetes mellitus), none of the measures of lipid profile were independently associated with C-IMT (table 3.8). Age was strongly and independently associated with C-IMT in all models ($p<0.0001$) and postmenopausal status was independently associated with C-IMT only in the models with LDL cholesterol ($p=0.0455$) or triglycerides ($p=0.0438$). In addition, in multivariate models which also included adjustments for other demographic factors associated with C-IMT in the literature (heart rate, current smoking, regular alcohol consumption and gender in addition to age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status and diabetes mellitus), none of the measures of lipid profile were independently associated with C-IMT (figure 3.14 and table 3.9). Similarly, when men and women were analysed separately, none of the measures of lipid profile were associated with C-IMT independent of age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status (women only), diabetes mellitus, heart rate, current smoking and regular alcohol consumption (figure 3.15, table 3.10 and table 3.11). Age was the strongest determinant of C-IMT in all participants (table 3.9), in men (table 3.10) and in women (table 3.11).

Table 3.8 Multivariate relationships between C-IMT and measures of lipid profile in all study participants (n=430).

	Partial r	95% confidence interval	p value
Total cholesterol	-0.027	-0.123 to 0.070	=0.590
HDL cholesterol	-0.066	-0.161 to 0.030	=0.179
LDL cholesterol	0.013	-0.083 to 0.110	=0.787
Triglycerides	-0.019	-0.115 to 0.078	=0.703
Total-to-HDL cholesterol ratio	0.033	-0.063 to 0.129	=0.499

Models included age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status and diabetes mellitus in addition to those indicated. Each of the measures of lipid profile were analysed in separate models.

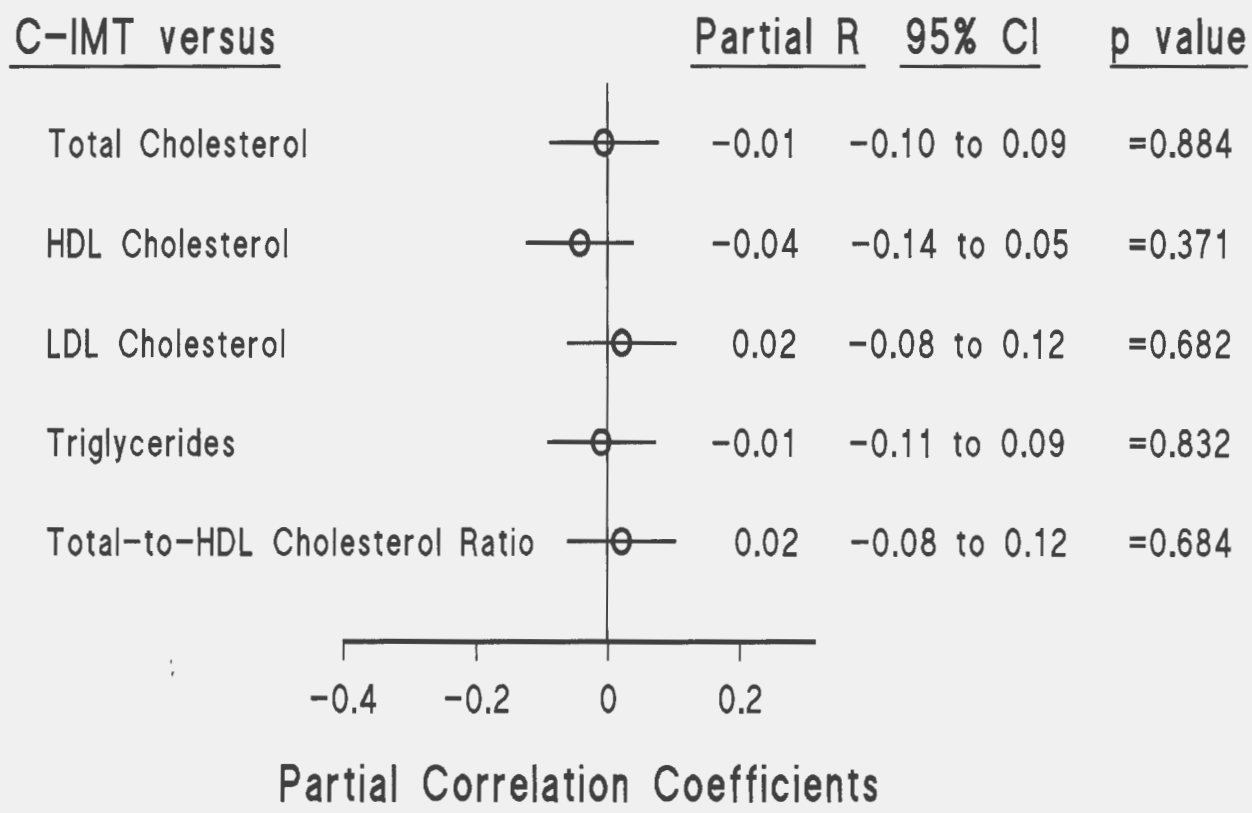

Figure 3.14 Multivariate relationships between C-IMT and measures of lipid profile in all participants. Age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status, sex, diabetes mellitus, heart rate, current smoking and regular alcohol consumption were included in the models.

Table 3.9 Multivariate relationships between C-IMT and measures of lipid profile in all study participants (n=430).

	Standardised β-coefficient ± SEM	p value
	Model with Total cholesterol	
Total cholesterol	-0.006 ± 0.042	=0.884
Age	0.660 ± 0.057	<0.0001
Female Sex	0.187 ± 0.092	=0.0424
	Model with HDL cholesterol	
HDL cholesterol	-0.035 ± 0.039	=0.371
Age	0.659 ± 0.055	<0.0001
Female Sex	0.187 ± 0.090	=0.0377
	Model with LDL cholesterol	
LDL cholesterol	0.017 ± 0.042	=0.682
Age	0.653 ± 0.056	<0.0001
Female Sex	0.197 ± 0.091	=0.0318
	Model with Triglycerides	
Triglycerides	-0.009 ± 0.041	=0.832
Age	0.660 ± 0.056	<0.0001
Female Sex	0.188 ± 0.090	=0.0374
	Model with Total-to-HDL cholesterol ratio	
Total-to-HDL cholesterol ratio	0.018 ± 0.043	=0.684
Age	0.654 ± 0.056	<0.0001
Female Sex	0.193 ± 0.090	=0.0328

Models included clinic systolic blood pressure, treatment for hypertension, postmenopausal status, diabetes mellitus, heart rate, current smoking and regular alcohol consumption in addition to those indicated. Each of the measures of lipid profile were analysed in separate models.

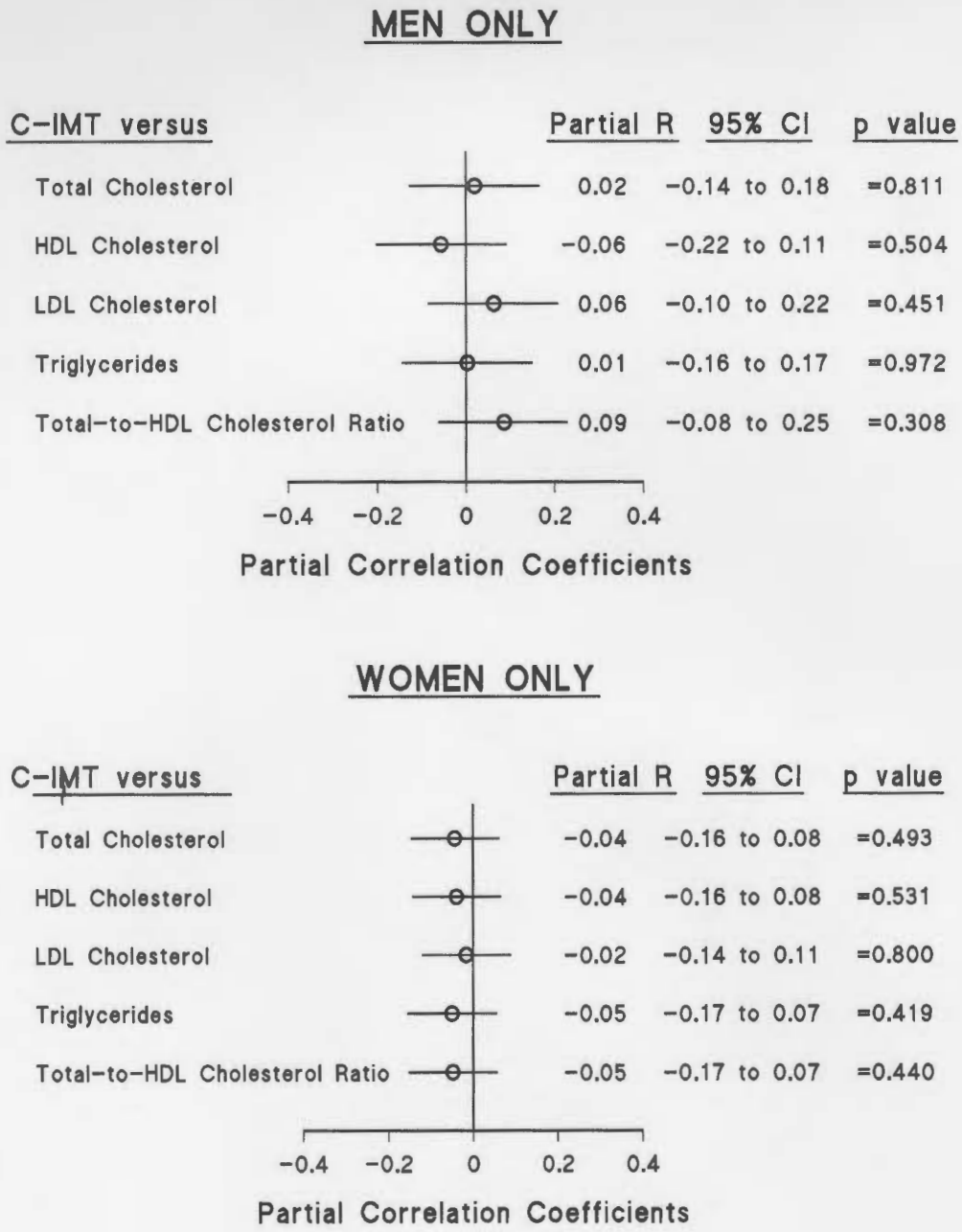

Figure 3.15 Multivariate relationships between C-IMT and measures of lipid profile in men and women separately. Age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status (women only), diabetes mellitus, heart rate, current smoking and regular alcohol consumption were included in the models.

Table 3.10 Multivariate relationships between C-IMT and measures of lipid profile in male study participants (n=152).

	Standardised β-coefficient ± SEM	p value
	Model with Total cholesterol	
Total cholesterol	0.016 ± 0.067	=0.811
Age	0.622 ± 0.090	<0.0001
	Model with HDL cholesterol	
HDL cholesterol	-0.044 ± 0.066	=0.504
Age	0.631 ± 0.085	<0.0001
	Model with LDL cholesterol	
LDL cholesterol	0.048 ± 0.064	=0.451
Age	0.613 ± 0.088	<0.0001
	Model with Triglycerides	
Triglycerides	0.002 ± 0.069	=0.972
Age	0.628 ± 0.089	<0.0001
	Model with Total-to-HDL cholesterol ratio	
Total-to-HDL cholesterol ratio	0.071 ± 0.069	=0.308
Age	0.607 ± 0.088	<0.0001

Models included clinic systolic blood pressure, treatment for hypertension, diabetes mellitus, heart rate, current smoking and regular alcohol consumption in addition to those indicated. Each of the measures of lipid profile were analysed in separate models.

Table 3.11 Multivariate relationships between C-IMT and measures of lipid profile in female study participants (n=278).

	Standardised β-coefficient ± SEM	p value
	Model with Total cholesterol	
Total cholesterol	-0.036 ± 0.067	=0.493
Age	0.576 ± 0.087	<0.0001
	Model with HDL cholesterol	
HDL cholesterol	-0.030 ± 0.048	=0.531
Age	0.566 ± 0.086	<0.0001
	Model with LDL cholesterol	
LDL cholesterol	-0.014 ± 0.054	=0.800
Age	0.570 ± 0.087	<0.0001
	Model with Triglycerides	
Triglycerides	-0.043 ± 0.053	=0.419
Age	0.569 ± 0.086	<0.0001
	Model with Total-to-HDL cholesterol ratio	
Total-to-HDL cholesterol ratio	-0.043 ± 0.056	=0.440
Age	0.572 ± 0.086	<0.0001

Models included clinic systolic blood pressure, treatment for hypertension, diabetes mellitus, heart rate, postmenopausal status, current smoking and regular alcohol consumption in addition to those indicated. Each of the measures of lipid profile were analysed in separate models.

3.7 Association of C-IMT with Anthropometric Data - Lipid Profile Interactive Terms

In bivariate analyses, each of the interactive terms was associated with C-IMT (table 3.12). However, in multivariate analyses adjusting for confounders (age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status and diabetes mellitus, heart rate, current smoking, regular alcohol consumption and gender) none of the interactive terms remained independently associated with C-IMT (table 3.13). In the multivariate models only age was strongly and independently associated with C-IMT ($p<0.0001$).

3.8 Association of C-IMT with Anthropometric Data and Measures of Lipid Profile in the Same Model

In multivariate analyses of each obesity index together with each measure of lipid profile, without adjusting for confounders, all indices of obesity ($p=0.016$ to $p<0.0001$) and measures of lipid profile ($p=0.0014$ to $p<0.0001$), except for HDL cholesterol ($p=0.88$ to $p=0.25$) were associated with C-IMT. However, in multivariate analyses after adjusting for confounders (age, clinic systolic blood pressure, treatment for hypertension, postmenopausal status and diabetes mellitus, heart rate, current smoking, regular alcohol consumption and gender) none of the obesity indices ($p=0.211$ to $p=0.904$) nor any of the measures of lipid profile ($p=0.283$ to $p=0.954$) remained independently associated with C-IMT. In these multivariate models only age was strongly and independently associated with C-IMT ($p<0.0001$).

Table 3.12 Bivariate relationships between C-IMT and interactive terms between anthropometric data and measures of lipid profile in all study participants (n=430).

	r	p value	r	p value	r	p value	r	p value	r	p value
	BMI		Waist circ.		Hip circ.		Skinfold thickness		Waist-to-hip ratio	
Total cholesterol	0.294	<0.0001	0.325	<0.0001	0.258	<0.0001	0.233	<0.0001	0.323	<0.0001
HDL cholesterol	0.105	=0.029	0.103	=0.034	-0.004	=0.938	0.116	=0.016	0.031	=0.516
LDL cholesterol	0.283	<0.0001	0.302	<0.0001	0.248	<0.0001	0.241	<0.0001	0.298	<0.0001
Triglycerides	0.280	<0.0001	0.273	<0.0001	0.252	<0.0001	0.248	<0.0001	0.278	<0.0001
Total-to-HDL cholesterol ratio	0.291	<0.0001	0.302	<0.0001	0.259	<0.0001	0.249	<0.0001	0.296	<0.0001

Each of the interactive measures were analysed in separate models. BMI, body mass index; Waist circ, waistc circumference; Hip circ, hip circumference.

Table 3.13 Multivariate relationships between C-IMT and anthropometric data and measures of lipid profile in the same model in all study participants (n=430).

	r	p value	r	p value	r	p value	r	p value	r	p value
	BMI		Waist circ.		Hip circ.		Skinfold thickness		Waist-to-hip ratio	
Total cholesterol	-0.005	=0.923	-0.031	=0.529	-0.013	=0.799	0.030	=0.546	-0.034	=0.490
HDL cholesterol	-0.033	=0.512	-0.056	=0.259	-0.038	=0.437	0.009	=0.858	-0.064	=0.190
LDL cholesterol	0.011	=0.829	-0.003	=0.958	0.010	=0.833	0.040	=0.420	0.0003	=0.995
Triglycerides	-0.007	=0.894	-0.035	=0.474	-0.021	=0.666	0.003	=0.951	-0.026	=0.596
Total-to-HDL cholesterol ratio	0.016	=0.746	-0.006	=0.904	0.008	=0.864	0.039	=0.431	-0.001	=0.982

Models included age, clinic systolic blood pressure, treatment for hypertension, diabetes mellitus, heart rate, postmenopausal status, current smoking, regular alcohol consumption and gender. Each of the interactive measures were analysed in separate models. BMI, body mass index; Waist circ, waistc circumference; Hip circ, hip circumference.

Chapter 4

Discussion

4.1 Primary outcomes of the present study

The primary finding in this study is that in an urban developing community of African ancestry (age range 16-86 yrs), despite a high prevalence of excess adiposity, there was no independent relationship between C-IMT and either indices of obesity or measures of lipid profile after adjustment for confounders. Only age and gender were found to independently predict C-IMT. These findings were consistent when men were analysed separately from women.

Although, in unadjusted (bivariate) analysis total cholesterol, triglycerides, LDL cholesterol and total:HDL cholesterol ratio (but not HDL cholesterol) were associated with C-IMT in the whole population; these relationships did not survive multivariate adjusted analysis. Similarly, although in unadjusted (bivariate) analysis, all indices of obesity [indices of general (BMI), abdominal (WHR) and subcutaneous (skinfolds) obesity] were associated with C-IMT; these relationships were not independent of confounding factors. In both instances age was the predominant factor influencing C-IMT. In the absence of confounders, indices of obesity and measurements of lipid profile were associated with C-IMT independent of each other; but neither survived the strong relationship between C-IMT and age.

These data indicate that in an urban developing community of African ancestry, in whom the risk factors for increased C-IMT are relatively low (predominant risk factors were obesity and hypertension; as the prevalence of increased lipids, diabetes mellitus and smokers was low), the impact of lipids and indices of obesity (irrespective of whether indices of general, abdominal or subcutaneous obesity were assessed) although independent of each other do not survive the major impact of advancing age. In keeping with the relatively low risk for increased C-IMT, only 8% of this population was noted to have C-IMT values above the 75th percentile for gender and age. Of interest the predominant risk factor, obesity; but also the

unfavourable lipid profiles were mainly observed in women which is in contrast to opposite gender differences observed in other ethnic groups (Raitakari *et al.* 2003).

4.2 Comparisons with previous studies

Consistent with previous literature, we found that measures of lipid profile (except for HDL cholesterol) were associated with C-IMT on bivariate analysis (Salonen & Salonen 1991; Ciccone *et al.* 2001; Davis *et al.* 2001; Baldassarre *et al.* 2002; Urbina *et al.* 2002; Raitakari *et al.* 2003; Mackinnon *et al.* 2004; Lo *et al.* 2006; Koskinen *et al.* 2009; Skilton *et al.* 2009; Paul *et al.* 2011). Although some studies noted an inverse relationship between HDL cholesterol and C-IMT (Davis *et al.* 2001; Baldassarre *et al.* 2002; Urbina *et al.* 2002; Koskinen *et al.* 2009; Skilton *et al.* 2009; Paul *et al.* 2011), similar to our study other studies (Salonen & Salonen 1991; Ciccone *et al.* 2001; Raitakari *et al.* 2003; Mackinnon *et al.* 2004; Lo *et al.* 2006; Maher *et al.* 2009) failed to show a relationship between HDL cholesterol and C-IMT. In addition, consistent with the literature, we found that all indices of obesity (indices of general, abdominal or subcutaneous obesity) were associated with C-IMT on bivariate analysis (Ciccone *et al.* 2001; Davis *et al.* 2001; Urbina *et al.* 2002; Raitakari *et al.* 2003; Mackinnon *et al.* 2004; Kotsis *et al.* 2006; Lo *et al.* 2006; Hassinen *et al.* 2007; Naya *et al.* 2008; Koskinen *et al.* 2009; Maher *et al.* 2009).

However, on multivariate analyses we were unable to show an independent relationship between either measures of lipid profile or any of the indices of obesity and C-IMT. In those studies which have performed multivariate analyses, some reported an impact of cholesterol on C-IMT independent of measures of obesity (Urbina *et al.* 2002; Sarmento *et al.* 2009; Paul *et al.* 2011); whereas others reported an impact of obesity (BMI and/or waist circumference,

WHR, skinfolds) on C-IMT independent of measures of lipid profile (Ciccone *et al.* 2001; de Michele *et al.* 2002; Lo *et al.* 2006; Koskinen *et al.* 2009; Maher *et al.* 2009; Skilton *et al.* 2009; Soliman *et al.* 2010). However, some of these studies either failed to adjust for age (Sarmento *et al.* 2009) which is the major determinant of C-IMT (Howard *et al.* 1993; Polak, 2009), only assessed young populations (20-43 yrs of age, table 1.1: Davis *et al.* 2001; Urbina *et al.* 2002; Raitakari *et al.* 2003; Paul *et al.* 2011; table 1.2: Ciccone *et al.* 2001, 18-45 yrs; Koskinen *et al.* 2009, mean age = 32 yrs; Lo *et al.* 2006, 24-59 yrs) or assessed a population with a narrow age range (40-60 yrs, Salonen & Salonen 1991; 30-69 yrs, de Michele *et al.* 2002); in which case age may be less likely to mask the effects of ether obesity or cholesterol. Only Lorenz *et al.* 2006 assessed participants over the full adult age range (19-90 yrs); however this study only performed bivariate analyses (no multivariate analyses were performed). Although in the study by Maher *et al.* 2009, the mean age was 41 years, participants were specifically selected on the basis of normal cholesterol and normal blood pressure values. Naya *et al.* 2008 assessed participants who ranged in age from 18-62 years but they were not able to show a relationship between age and C-IMT. Only one study (Skilton *et al.* 2009) assessed participants over the full adult age range (21-83 yrs); however in this study the maximum BMI was only 28 kg/m^2 and approximately 40% of participants were receiving lipid lowering agents. Hence, these studies did not adequately address the possible independent impact of obesity and/or cholesterol on C-IMT at a population level.

Moreover, in many of the studies showing an effect of cholesterol independent of obesity, mean BMI was not in the obese range (28 kg/m^2, Urbina *et al.* 2002; ~29 kg/m^2, Paul *et al.* 2011) and hence it is less likely that obesity would mask the effects of cholesterol. Indeed, Hassinen *et al.* 2007, in a 12 year follow-up study in which the participants had a mean BMI of 26 kg/m^2, reported that obesity does not impact on C-IMT independent of cholesterol concentrations. Moreover, many studies showing an independent effect of cholesterol on C-

IMT, were conducted in subjects with hypercholesterolemia who were not obese (mean BMI 24 $kg.m^{-2}$, Gariepy *et al.* 1995; mean BMI 26 $kg.m^{-2}$, Wittekoek *et al.* 1999), which would have enhanced the impact of cholesterol.

Similarly, in many of the studies showing an effect of obesity independent of cholesterol, mean cholesterol concentrations were not particularly high (4.2 mol/l, Ciccone *et al.* 2001; 4.53 mmol/l, Lo *et al.* 2006; 5.1 mmol/l, Koskinen *et al.* 2009; 5.0 mmol/l, Maher *et al.* 2009; 5.04 mmol/l, Soliman *et al.* 2010) or a large proportion (~40%) of participants were receiving lipid lowering therapy (Skilton *et al.* 2009) and hence cholesterol effects would be less likely to mask the effects of obesity. In this regard it is important to note that in our study although the prevalence of overweight and obesity was high, we did not show an independent effect of obesity on C-IMT, despite the low cholesterol concentrations.

Consistent with the low cholesterol concentrations noted in our study, current literature shows that obese black women have low total and LDL cholesterol concentrations ($p<0.05$) compared to obese white women after adjusting for age (Goedecke *et al.* 2010). Urbina *et al.* 2002, reported similar ethnic differences in both men and women, and Omran, 2005, reported that people of African ancestry have low total cholesterol concentrations. In addition, significant ethnic differences were observed for triglyceride and HDL cholesterol levels, with black participants having lower triglyceride and higher HDL cholesterol levels compared to white participants (Srinivasan *et al.* 1976; Paul *et al.* 2011). Of interest was the observation of a greater prevalence of obesity and unfavourable lipid profiles in women compared to men in people of African ancestry. In comparison, in other ethnic groups men are at a greater risk (Raitakari *et al.* 2003). The reasons for these gender-ethnic differences are presently unknown. It is possible that lifestyle and lack of physical activity may play a role. Nevertheless, multivariate analysis in gender specific groups in essence revealed the same

outcomes in our study.

With regard to factors other than measures of obesity and lipid profile, consistent with previous literature (Salonen & Salonen 1991; Ciccone *et al.* 2001; Davis *et al.* 2001; Lo *et al.* 2006; Naya *et al.* 2008; Koskinen *et al.* 2009; Maher *et al.* 2009), we were able to show that age, gender, systolic and diastolic BP, and diabetes mellitus were associated with C-IMT on bivariate analyses. Moreover, our data after multivariate analysis confirm that age is the major determinant of C-IMT (Howard *et al.* 1993; Ciccone *et al.* 2001; Koskinen *et al.* 2009; Polak, 2009).

4.2 Proposed potential mechanisms of effects of age, obesity and lipids on C-IMT

The proposed mechanisms by which age affects C-IMT are that endothelial cells age which leads to endothelial dysfunction resulting in increased permeability of the vascular membrane. Hence, lipoproteins circulating in the blood enter the vascular wall resulting in lipid deposits within the intima and media. In addition, with increasing age, fibrosis is increased and smooth muscle cells proliferate which further contributes to the increase in intima-media thickness (Carallo *et al.* 1999; Erusalimsky *et al.* 2009). Luo *et al.* 2011 found that the left C-IMT showed no obvious change before the age of 35 years, and after the age of 35 it thickened considerably every 10 years. Moreover, in a study of participants ranging in age from 45 to 64 years, it was found that the rate by which C-IMT increases is approximately 0.010 mm/yr in both men and women (Howard *et al.*1993). Furthermore, the rate of change of C-IMT is reported to be in the range of 0.008 to 0.0147 mm/yr, such that 0.1 mm translates into an expected change over 7 to 10 years (Polak, 2009). Hence age is an important parameter or risk factor of C-IMT progression.

In terms of the proposed mechanisms of the effects of obesity on C-IMT; some studies have linked obesity to an increase in arterial wall thickness by proposing that leptin may be important for the regeneration of endothelial intimal layer after vascular injury (Correia & Haynes, 2001). In an experiment involving leptin receptor-deficient *db/db* mice it was found that neointimal formation was substantially reduced by 90% after experimental endovascular injury when compared to leptin-sensitive wild-type mice (Stephenson *et al.* 2003). Many studies have shown women to have higher leptin concentrations than men (Ciccone *et al.* 2001; Considine, 2001); however this would argue against the higher C-IMT values reported in men compared to women (Howard *et al.* 1993; Urbina *et al.* 2002; Raitakari *et al.* 2003; Paul *et al.* 2011). Hence, the mechanisms of the effects of obesity on C-IMT need to be investigated further.

The mechanisms of the effects of cholesterol on C-IMT are well established. As discussed in my introduction (section 1.2); one of the primary factors responsible for increases in arterial wall thickness is an increase in LDL cholesterol (Poredos 2004; Beckett *et al.* 2000), which plays an important role in the initiation and progression of atherosclerosis (Naito *et al.* 1994; Jia *et al.* 2009). The subsequent accumulation of lipid and fibrosis within the intima and media of the arterial wall results in the increase in C-IMT.

4.3 Brief background on epidemiology of cardiovascular disease to indicate the uniqueness of populations of African ancestry

When we view the health of human beings tracing back from history in the earliest nomadic times, sickness and early death were mainly the result of food deficiency. Thinly stretched populations lessened the impact of infectious diseases. Only about 10,000 years ago animals were domesticated and people discovered how to cultivate nutritious plants and harvest them, hence making food more plentiful and encouraging population growth. The resultant

crowding made infectious disease somewhat more prevalent and nutrition a strong influence on health. In developed countries, this transitional period began 300 years ago with industrial and transportation developments, which encouraged progressive urbanization (Keil & Saunders, 1991).

When the industrial revolution enticed people from farming areas to urban centers, they were at increased risk of disease and death because they were forced to live in close proximity to each other, making transmission of disease easier. Tuberculosis, smallpox, typhoid fever, and other infectious diseases were rampant. With the advent of the golden age of medicine, immunizations, improved hygiene, and the use of antibiotic therapy, infectious diseases declined markedly and the chronic diseases namely the cardiovascular diseases and cancer began to show an increase (Keil & Saunders, 1991). This increase has been attributed to individuals surviving one kind of disease to be able to confront another kind. In addition, lifestyles changed in the period up to the mid-1960s; cigarette smoking increased, sedentary work and leisure time was aspired to, and obesity became more prominent (Keil & Saunders, 1991). Dairy products and eggs, which had been initially advocated to provide dietary protein, were used in increasing quantities, and as the industrial and agricultural economies prospered, the consumption of saturated fats increased (Keil & Saunders, 1991).

Evidence of the impact of such economic transitions is provided by a study conducted in Georgia, USA. During 1960 to 1962, the Evans County (Georgia) found lower coronary heart disease (CHD) prevalence and incidence rates among black compared to white males even after controlling for standard CHD risk factors (Cassel *et al.* 1971). The prevailing view through the 1970s was that CHD is uncommon among black individuals (Gillum & Liu, 1984). More recent evidence has dispelled these early views taking into account changes in economic and environmental circumstances, and it is now recognized that CHD mortality

rates for African Americans exceed those of whites aged 25 to 64 years, and are similar to the rates of whites for all ages combined (Gillum, 1982; Watkins, 1984; Department of health and human services, 1985). Indeed, the differences in HDL cholesterol concentrations generally reported in African American men compared to Caucasian men (African Americans have higher HDL cholesterol concentrations), are not evident in men at higher educational levels (Glueck *et al.* 1984; Watkins *et al.* 1986).

However, in Africa as a whole, the commencement of this transition to urbanization has been slow and indeed has not progressed as rapidly as in the rest of the world (Keil & Saunders, 1991). In many countries in sub-Saharan Africa, the above transitions areas are still occurring. Hence, in sub-Saharan Africa the consumption of saturated fats is minimal, particularly in people living in more rural areas, thus resulting in low cholesterol levels in populations of African ancestry. In South Africa, only in the recent past (since 1994), has there been a marked increase in the number of individuals migrating from rural to urban areas; hence cholesterol levels are still low in populations of African ancestry. However, the increase in the prevalence of obesity within these communities, suggest that they are currently undergoing economic transition. Hence, if measures are not taken to prevent overweight and obesity as well as possible increases in cholesterol concentrations, it is likely that in time CHD mortality rates for blacks will exceed those of whites similar to what happened in Georgia, USA in the 1980's (Gillum *et al.*1982; Watkins, 1984; Department of health and human services, 1985).

Obesity is, by far the most pervasive diet-related risk factor for CVD among African American adults both because it relates to so many different aspects of CVD and because obesity occurs with high frequency in the black community (Kumanyika & Adams-Campbell, 1991). Data from the center for Health Statistics in 1987 showed that black women have

larger sub scapular skinfold thickness than white women and/or men at age 30, 50 and 70 years. It was also recorded that African American males have higher sub scapular skinfold thickness than Caucasian men in the same middle-age range (35 to 54 years) in which overweight prevalence is higher in African American than Caucasian men (Kumanyika & Adams-Campbell, 1991). Some evidence suggests that lower levels of energy expenditure rather than higher levels of intake may be a critical factor predisposing women of African ancestry to high levels of obesity. In the 1985 National Center for Health Statistics (NHIS) data (1988), black women were less likely than white women to report engaging in regular exercise or sports activity. In South Africa, the prevalence of obesity has increased dramatically over the past few years. Indeed, recent studies report approximately 60% of populations of African ancestry to be overweight or obese (Steyn *et al.* 2005; Majane *et al.* 2007; Norton *et al.* 2009).

It should be noted that South Africa is a developing country; hence people of African ancestry are still going through an epidemiologic transition (Omran, 2005; Sliwa *et al.* 2008; Stewart *et al.* 2008). Therefore people in Sub-Saharan Africa have a diet that is low in cholesterol thus resulting in lower total cholesterol concentrations as compared to Caucasians and African Americans (Connor *et al.* 2009). In a population of African ancestry there is a low incidence of atherosclerosis compared to populations of Caucasian ancestry (Bensen *et al.* 1999; Connor *et al.* 2009). Hence, the main factor that could contribute to the difference in the findings in our study compared to those of previous studies is the fact that people of African ancestry are still going through an epidemiologic transition and hence they still have low total cholesterol levels.

4.3 The cardiovascular pathophysiology of people of African ancestry

Cardiovascular disease in people of European descent is mainly attributed to classical risk factors, namely diets high in saturated fats, elevated serum cholesterol concentrations, high blood pressure, diabetes mellitus, and smoking. However, among African Americans in America, the rates of CVD are comparable or higher than the rates among Caucasians in America (Yusuf *et al.* 2001). Nevertheless, the incidence of cardiovascular disease in people of African ancestry in Africa is still relatively low when compared to rates in most western countries. Many studies explaining the pathophysiology of cardiovascular disease attribute it to the high incidence of hypertension in the African community. The multiple factors that are associated with hypertension in populations of African ancestry include low plasma renin, sodium abnormalities, epithelium sodium channel changes, altered changes regulating the renin-angiotensin-aldosterone system (RAAS), increased peripheral vascular resistance, increasing obesity, and socio-economic status (Opie & Seedat, 2005). Populations of African ancestry are salt-sensitive and in the presence of high sodium intake plasma renin is suppressed. The above mentioned factors also occur in African Americans. Compared with Caucasians, African-Americans develop hypertension at an earlier age. The reason for black-white differences in hypertension prevalence likely involves a complex interaction between environmental response to diet, stress, and a potential genetic/physiological difference in sodium/potassium excretion (Yusuf *et al.* 2001). We now know that people of African ancestry have a problem of sodium retention. The major factor controlling sodium excretion is renal perfusion pressure as it drives glomerular filtration since causes of hypertension are mediated through the kidney. Any processes that lead to a decrease in this pressure will lead to decreased sodium filtration and increases reabsorption. Anatomic changes in the renal vasculature can also lead to a decreased renal perfusion pressure (Blaustein & Grim, 1991). Some studies have attributed the increase in C-IMT to shear walls stress due to the curvature

of the carotid artery through studies of fluid mechanics (Caro *et al.* 1996; Caro, 2009). These studies have shown that as blood flows through the curvature of the carotid artery vascular injury is caused through shear wall stress which leads to atherosclerosis. In our study we did not determine whether the RAAS may affect C-IMT, therefore future studies should investigate whether the RAAS influences C-IMT in this population.

4.4 Limitations

A possible limitation of our study was the low sample size of men compared to women. As C-IMT is generally higher in men compared to women (Howard *et al.* 1993; Urbina *et al.* 2002; Paul *et al.* 2011), this sample size may have limited our ability to detect independent effects of obesity and/or cholesterol on C-IMT. However, in our study, no gender differences in C-IMT were evident. This may be due to the fact that more women were obese, and that the women had higher cholesterol concentrations than the men. Indeed, in studies reporting greater C-IMT in African American men versus women, the men had higher cholesterol concentrations and blood pressures compared to the women (Urbina *et al.* 2002; Paul *et al.* 2011).

4.5 Conclusions and perspectives

Therefore, in conclusion there was no major independent relationship between either circulating lipid concentrations or obesity and atheroma (as indexed by C-IMT) in an urban developing community of African ancestry. The data from our study suggest that urban, developing communities of African ancestry in South Africa are in an early phase of a population health transition. We are not disputing the fact that increases in cholesterol levels cause atheroma; however at this stage cholesterol levels are too low to produce a large impact on atheroma in the African population. At present age is more important at predicting an

increase in C-IMT in an urban developing community of African ancestry. More importantly we have to take into consideration that the migration of people of African ancestry from rural to urban settings has affected their diet, and now they may be consuming products with high total cholesterol content. Hence increased cholesterol concentrations may be a major CVD risk factor in the future. Indeed, once people of African ancestry develop high total cholesterol levels, similar to African Americans, they may even be at a higher risk than Caucasians due to the high incidence of obesity (mainly in women) and untreated hypertension. Hence our findings are very important in the context that this data may be able to assist the current South African government to be proactive rather than reactive in educating the population at large in terms of change in lifestyle, better nutrition and more access to health services such as yearly medical check-ups especially with the proposed national health insurance (NHI).

4.6 Ideas for future research

Our finding of a lack of independent association between induces of obesity and C-IMT in a population of African ancestry, despite a very high incidence of obesity (41%) deserves some further comment. In this regard, a number of studies have shown genetic differences between populations of different race or ethnicity. With respect to genetic polymorphisms associated with LDL cholesterol, the apolipoprotein E2 allele which is positively associated with LDL cholesterol concentrations, has a greater impact on lipid levels in Caucasians compared to African Americans, despite a higher prevalence of this polymorphism in African Americans (Pablos-Mendez *et al.* 1997). The lack of effect of the apolipoprotein E2 allele on lipid levels and the incidence of CHD in African Americans has been attributed to the protective effects of dietary and/or lifestyle effects in this ethnic group (Loktionov *et al.* 1999). In addition, the genetic determinants of lipids may differ between different race and ethnic groups. Indeed, Chang *et al.* (2010) showed that although variants of the apolipoprotein E and *ITGB3*

(integrin β3 or platelet glycoprotein IIIa) genes were associated with LDL and total cholesterol and triglycerides in non-Hispanic blacks, these variants showed no association in non-Hispanic whites. Similarly, although a *PON1* (paraoxonase I, which plays a role in oxidative stress) gene variant was associated with LDL and total cholesterol in non-Hispanic whites, this variant showed no association in non-Hispanic blacks (Chang *et al.* 2010). With respect to genetic polymorphisms associated with obesity, although the increased prevalence of obesity in blacks has been explained by the increased frequency of the 825T allele of the G protein β3 subunit gene; the lack of effect of obesity on CVD risk has been suggested to be due to possible gene-environmental interactions (Siffert *et al.* 1999). Indeed, Predazzi *et al.* (2010) attribute some of the varying susceptibility to CVD in different ethnic groups to different allele frequencies and gene-environmental interactions. Hence, in order to understand the current apparent protection of populations of African ancestry despite the higher prevalence of obesity, future research should focus on possible gene-environmental interactive effects on C-IMT and CVD risk in populations of African ancestry.

Chapter 5

References

1) Arnett DK, Tyroler HA, Burke G, Hutchinson R, Howard G *et al.* (1996). Hypertension and subclinical carotid artery atherosclerosis in blacks and whites: the Artherosclerosis Risk in Communities Study: ARIC investigators. *Arch Intern Med* **156**, 1983-1989.

2) Baldassarre D, Amato M, Pustina L, Tremoli E, Sirtori CR *et al.* (2002). Increased carotid intima-media thickness in subjects with primary hypoalphalipoproteinemia. *Arterioscler Thromb Vasc Biol* **22**, 317-322.

3) Bamba V & Rader DJ (2007). Obesity and Atherogenic Dyslipidemia. *Gastroenterology* **132**, 2181-2190.

4) Beckett N, Nunes M & Bulpitt C (2000). Is it advantageous to lower cholesterol in the elderly hypertensive? *Cardiovasc Drugs Ther* **14**, 397- 405.

5) Belcaro G, Nicolaides AN, Ramaswami G, Cesaroni MR, Sanctis MD *et al.* (2001). Carotid and femoral ultrasound morphology screening and cardiovascular events in low risk subjects: a 10-year follow-up study (the CAFES-CAVE study). *Atherosclerosis* **156**, 379-387.

6) Bennett CM, Guo M & Dharmage SC (2007). HbA1c as a screening tool for the detection of type 2 diabetes: a systematic review. *Diabet Med* **24**, 333-343.

7) Bensen JT, Li R, Hutchinson RG, Province MA, & Tyroler HA (1999). Family history of coronary heart disease and pre-clinical carotid artery atherosclerosis in African Americans and whites: The ARIC study. *Genet Epidemiol* **16**, 165-178.

8) Blaustein MP & Grim CE (1991). The pathogenesis of hypertension: Black-White differences. In: Elijah Saunders & Brest AN, editors. *Cardiovascular diseases in blacks. Philadelphia*, 97-114.

9) Bonithon-kopp C, Toubol P, Berr C, Leroux C, Mainard F *et al.* (1996). Relation of intima-media thickness to atherosclerotic plaques in carotid arteries. *Arter, Thromb and vasc biol* **16**, 310-316.

10) Bots M, de Jong PT, Hofman A & Grobbee DE (1997). Left, right, near or far wall common carotid intima-media thickness measurements: Associations with cardiovascular disease and lower extremity arterial atherosclerosis. *J Clin Epidemiol* **50**, 801-807.

11) Bradshaw D, Groenewald P, Laubscher R, Nannan N, Nojilana B *et al.* (2003). Initial burden of disease estimates for South Africa, 2000. Cape Town: *South African Medical Research Council.*

12) Bray GA (2004). Medical consequences of obesity. *J Clin Endocrinol Metab* **89**, 2583-2589.

13) Caro CG, Doorly DJ, Tamawski M, Scott KT, Long Q *et al.* (1996). Non-planar curvature and branching of arteries and non-planar-type flow. *Proc. R. Soc. Lond. A* **452**, 185-197.

14) Caro CG (2009). Discovery of the role of wall shear in atherosclerosis. *Arterioscler Thromb Vasc Biol* **29**, 158-161.

15) Carallo C, Irace C, Pujia A, Franceschi MS, Crescenzo A *et al.* (1999). Evaluation of common carotid hemodynamic forces: Relations with wall thickening. *Hyper* **34**, 217-221.

16) Casella IB, Presti C, Porta RM, Sabbag RD, Bosch MA *et al.* (2008). A practical protocol to measure common carotid artery intima-media thickness. *Clinics* **64**, 515-520.

17) Cassel J, Heyden S, Bartel AG, Kaplan BH, Tyroler HA *et al.* (1971). Inicidence of coronary heart disease by ethnic group, social class, and sex. *Arch Intern Med* **128**, 901-906.

18) Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC *et al.* (1977). HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. *Circulation* **55**, 767-772.

19) Castelli WP, Wilson PWF, Levy D & Anderson K (1989). Cardiovascular risk factors in the elderly. *Am J Cardiol* **63**, 12-19.

20) Chang M, Yesupriya A, Ned RM, Mueller PW & Dowling NF (2010). Genetic variants associated with fasting blood lipids in the U.S. population: Third National Health and Nutrition Examination Survey. *BMC Medical Genetics* **11**, 1-13.

21) Chien K, Su T, Jeng J, Hsu H, Chang W, Chen M *et al.* (2008). Carotid artery Intima-media thickness, carotid plaque and coronary heart disease and stroke in Chinese. *PloS one* **3,** 1-8.

22) Ciccone M, Vettor R, Pannacciulli N, Minenna A, Bellacicco M *et al.* (2001). Plasma leptin is independently associated with the intima-media thickness of the common carotid artery. *Int J Obes* **25**, 805-810.

23) Cobble M & Bale B (2010). Carotid Intima-media thickness: Knowledge and application to everyday practice. *Postgraduate medicine* **122**, 7-14.

24) Connor MD, Modi G & Warlow CP (2009). Differences in the nature of stroke in a multiethnic urban South African population: the Johannesburg Hospital Stroke Register. *Stroke* **40**, 355-362.

25) Considine RV (2001). Regulation of leptin production. *Rev Endocr Metab Disord* **2**, 357-363.

26) Correia ML & Haynes WG (2004). Leptin, obesity and cardiovascular disease. *Curr Opin Nephrol Hypertens* **13**, 215-223.

27) Clement DL, De Buyzere ML, De Bacquer DA, de Leeuw PW, DuPrez DA, Fagard RH *et al.* (2003).Office versus ambulatory pressure study investigators. Prognostic value of ambulatory blood-pressure recordings in patients with treated hypertension. *N Engl J Med* **348**, 2407-2415.

28) Davis PH, Dawson JD, Riley WA & Lauer RM (2001). Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: The Muscatine Study. *Circulation* **104**, 2815-2819.

29) De Backer G, Ambrosioni E, Borch-Jonsen K, Brotons C, Cifkova R *et al.* (2003). European guidelines on cardiovascular disease prevention in clinical practice: Third Joint Task Force of European and other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of eight societies and by invited experts). *Eur Heart J* **24**, 1601-1610.

30) Dechering DG, Wizner B, Adiyaman A, Nawrot T, Jin Y, Richart T *et al.* (2009). Sphygmomanometric and ambulatory blood pressures as forerunners of carotid and femoral intima-media thickness. *J Hypertens.* **27**, 813-21.

31) de Groot E, Hovingh GK, Wiegman A, Duriez P, Smit AJ *et al.* (2004). Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. *Circulation* **109**, **III**-33-38.

32) Department of Health and Human Services (1985). Report of the Secretary's Task Force on Black and Minority Health. *US Government Printing Office, Washington.*

33) D'Agostino RB, Burke G, O'leary D, Rewers M, Selby J *et al.* (1996). Ethnic differences in carotid wall thickness: the Insulin Resistance Atherosclerosis study. *Stroke* **27**, 1744-1749.

34) Ebrahim S, Papacosta O, Whincup P, Wannamethee G, Walker M, Nicotaides AN *et al.* (1999). Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women. *Stroke* **30**, 841-850.

35) Erusalimsky JD (2009). Vascular endothelial senescence: from mechanisms to pathophysiology. *J Appl Physiol* **106**, 326-332.

36) Freedman DS, Katzmarzyk PT, Dietz WH, Srinivasan SR & Berenson GS (2009). Relation of body mass index and skinfold thicknesses to cardiovascular disease risk factors in children: The Bogalusa Heart Study[1-4]. *Am J Clin Nutr* **90**, 210-216.

37) Garaulet M, Perez-Lamas F, Baraz JC, Garcia-Prieto MD, Fardy PS, Tebar FJ *et al.* (2002). Body fat distribution in pre-and post-menopausal women: metabolic and anthropometric variables. *J Nutr Health Aging* **6**, 123-126.

38) Gariepy J, Simon A, Massonneau M, Linhart A & Levenson J (1995). Wall thickening of carotid and femoral arteries in male subjects with isolated

hypercholesterolemia. PCVMETRA group. Prevention CardioVasculaire en Medecine du Travail. *Atherosclerosis* **2**, 141-151.

39) Gaziano JM, Hennekens CH, O'Donnell CJ, Breslow JL & Buring JE (1997). Fasting triglycerides, high-density lipoprotein, and risk of myocardial infarction. *Circulation* **96**, 2520-2525.

40) Gillum RF (1982). Coronary heart disease in black populations. I. Mortality and morbidity. *Am Heart J* **104**, 839-851.

41) Gillum RF & Liu KC (1984). Coronary heart disease mortality in the United States blacks, 1940-1978: Trends and unanswered questions. *Am Heart J* **108**, 728-732.

42) Ginsberg HN, Zhang YL & Hernandez-Ono A (2006). Metabolic syndrome: focus on dyslipidemia. *Obesity (Silver Spring)* **14**, S41-S49.

43) Glueck CJ, Gartside P, Laskarzewski PM, Khoury P & Tyroler HA (1984). High-density lipoprotein cholesterol in blacks and whites: Potential ramifications for coronary heart disease. *Am Heart J* **108**, 815-826.

44) Goedecke JH, Utzschneider K, Faulenbach MV, Rizzo M, Berneis K *et al.* (2010). Ethnic differences in serum lipoproteins and their determinants in South African women. *J Metabol* **59**, 1341-1350.

45) Hamer M, Malan L, Schutte AE, Huisman HW, van Rooyen JM *et al.* (2011). Conventional and behavioral risk factors explain differences in sub-clinical vascular disease between black and Caucasian South Africans: The SABPA study. *Atherosclerosis* **215**, 237-242.

46) Hassinen M, Lakka TA, Komulainen P, Haapala I, Nissien A & Rauramaa R (2007). Association of waist and hip circumference with 12-year progression of carotid intima-media thickness in elderly women. *Int J Obes* **31**, 1406-1411.

47) Howard G, Sharrett AR, Hiss G, Evans GW, Chambless LE *et al.* (1993). Carotid artery intimal-medial thickness distribution in general populations as evaluated by B-mode ultrasound. ARIC Investigators. *Stroke* **24**, 1297-1304.

48) Howard G, Manolio TA, Burke GL, Wolfson SK, O'Leary DH *et al* (1997). Does the association of risk factors and atherosclerosis change with age? An analysis of the combined ARIC and CHS cohorts. *Stroke* **28** 1693-1701.

49) Jia X, Wei M, Fu X, Gu X, Fan W, Zhang J *et al.* (2009). Intensive cholesterol-lowering therapy improves large artery elasticity in acute myocardial infarction patients. *Heart vessels* **24**, 340-346.

50) Keil JE & Saunders DE (1991). Urban and rural differences in cardiovascular disease in blacks. In: Elijah Saunders & Brest AN, editors. *Cardiovascular diseases in blacks. Philadelphia*, 17-44.

51) Koskinen J, Kahonen M, Viikari J, Taittonen L, Laitinen T,Ronnemaaa T *et al.* (2009). Conventional cardiovascular risk factors and metabolic syndrome in predicting carotid intima-media thickness progression in young adults: The Cardiovascular Risk in Young Finns Study. *Circulation* **120**, 229-236.

52) Kotsis VT, Stabouli SV, Papamichael CM & Zakopoulos NA (2006). Impact of obesity in intima media thickness of carotid arteries. *Obesity* **14**, 1708-1715.

53) Kumanyika S & Adams-Campbell LL (1991). Obesity, diet, and psychosocial factors contributing to cardiovascular disease in blacks. In: Elijah Saunders & Brest AN, editors. *Cardiovascular diseases in blacks. Philadelphia*, 47-73.

54) Lakka TA, Lakka H, Salonen R, Kaplan GA & Salonen JT (2001). Abdominal obesity is associated with accelerated progression of carotid atherosclerosis in men. *Atherosclerosis* **154**, 497-504.

55) Lerner DJ & Kannel WB (1986). Patterns of coronary heart disease morbidity and mortality in the sexes: A 26-year follow-up of the Framingham population. *Am Heart J* **111**, 383-390.

56) Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S *et al.* (2006). Diet and Lifestyle Recommendations Revision 2006 A scientific statement from the American Heart Association Nutrition Committee. *Circulation* **114**:82-96.

57) Lo J, Dolan SE, Kanter JR, Hemphill LC, Connelly JM, Lees RS *et al.* (2006). Effects of obesity, body composition, and adiponectin on carotid intima-media thickness in healthy women. *J Clin Endocrinol Metab* **91**, 1677-1682.

58) Loktionov A, Vorster H, O'Neill IK, Nell T, Bingham SA *et al.* (1999). Apolipoprotein E and methylenetetrahydrofolate reductase genetic polymorphisms in relation to other risk factors for cardiovascular disease in UK Caucasians and Black South Africans. *Atherosclerosis* **145**, 125-135.

59) Lopez AD, Mathers CD, Ezzati M, Jamison DT & Murray CJ (2006). Global and regional burden of disease and risk factors, 2001: systemic analysis of population health data. *Lancet* **367**, 1747-57.

60) Lorenz MW, von Kegler S, Steinmetz H, Markus HS & Sitzer M (2006). Carotid intima-media thickening indicates a higher vascular risk across a wide range: Prospective Data from the Carotid Atherosclerosis Progression Study (CAPS). *Stroke* **37**, 87-92.

61) Luo X, Tang Y, Cao T & Li Z (2011). Differences in left and right carotid intima-media thickness and the associated risk factors. *Clinical Radiology* **66**, 393-398.

62) Mackinnon AD, Jerrard-Dunne P, Sitzer M, Buehler A, von Kegler S *et al.* (2004). Rates and determinants of site-specific progression of carotid artery intima-media thickness: The Carotid Atherosclerosis Progression Study. *Stroke* **35**, 2150-2154.

63) Maher V, O'Dowd M, Carey M, Markham C, Byrne A, Hand E *et al.* (2009). Association of central obesity with early carotid intima-media thickening is independent of that from other risk factors. *Int J Obes* **33**, 136-143.

64) Majane OHI, Norton GR, Maseko M, Makaula S, Crowther N *et al.* (2007). The association of waist circumference with ambulatory blood pressure is independent of alternative adiposity indices. *J Hypertens* **25**, 1798-1806.

65) Majane OHI, Woodiwiss AJ, Maseko MJ, Crowther N, Dessein P &Norton GR (2008). Impact of age on the independent association of adiposity with pulse wave velocity in a population sample of African ancestry. *Am J Hypertens* **21**, 936-942.

66) Manson JE, Colditz GA, Stampfer MJ, Willett WC, Rosner B *et al.* (1990). A prospective study of obesity and risk of coronary heart disease in women. *N Engl J Med* **322**, 882-889.

67) Manson JE, Willett WC, Stampfer MJ, Colditz GA, Hunter DJ *et al.* (1995). Body weight and mortality among women. *N Engl J Med* **333**, 677–685.

68) Maseko M, Majane HOI, Milne JF, Norton GR & Woodiwiss AJ (2006). Salt intake in an urban, developing South African community. *Cardiovasc J of South Africa* **17**, 186-191.

69) Maseko MJ, Norton GR, Majane OH, Molebatsi N & Woodiwiss AJ (2011[a]). Global cardiovascular risk profiles of untreated hypertensives in an urban, developing community in Africa. *Cardiovasc J Afr* **22**, 261-267.

70) Maseko MJ, Woodiwiss AJ, Majane OHI, Molebatsi N & Norton GR (2011[b]). Marked underestimation of blood pressure control with conventional versus ambulatory measurements in an urban, developing community of African ancestry. *Am J Hypertens* **24**, 789-795.

71) Mendelsohn ME & Karas RH (1999). The protective effects of estrogen on the cardiovascular system. *New Engl J Med* **340**, 1801-1811.

72) McQuillan BM, Beilby JP, Nidorf M, Thompson PL & Hung J (1999). Hyperhomocysteinemia but not the C677T mutation of methylenetetrahydrofolate reductase is an independent risk determinant of carotid wall thickening: The Perth Carotid Ultrasound Disease Assessment Study (CUDAS). *Circulation* **99**, 2383-2388.

73) Michele MD, Salvatore P, Iannuzzi A, Celentano E, Ciardullo AV *et al.* (2002). Association of Obesity and Central Fat Distribution With Carotid Artery Wall Thickening in Middle-Aged Women. *Stroke* **33**, 2923-2928.

74) Miller GJ & Miller NE (1975). Plasma-high-density-lipoprotein concentration and development of ischemic heart-disease. *The lancet* **305**, 16-19.

75) Naito M, Yamada K, Hayashi T, Asai K, Yoshimine N *et al.* (1994). Comparative toxicity of oxidatively modified low-density lipoprotein and lysophosphatidylcholine in cultured vascular endothelial cells. *Heart Vessels* **9**, 183-187.

76) National Center for Health Statistics, Najjar MF, Rowland M & Rowland M(1987): Anthropometric reference data and prevalence of overweight , United States, 1976-1980Vital and Health Statistics. *Series* **11**, *No* 238 *DHHS Pub No (PHS)* 87-1688. *US Government Printing Office, Washington.*

77) National Center for Health Statistics, Schoenborn CA (1988): Health and disease prevention: United states, 1985. *Vital and Health statistics, series* **10**, *No.* 163, *DHHS Pub No (PHS)* 88-1591, *Public Health Service. US Government Printing Office, Washington.*

78) National Institutes of Health, National heart, Lung, and blood institute (1998). Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: the evidence report. *Obes Res* **6**, S51-S210.

79) Naya T, Hosomi N, Ohyama H, Ichihara S, Ban CR *et al.* (2008). Smoking, fasting serum insulin and obesity are predictors of carotid atherosclerosis in relatively in young subjects. *Angiology* **58**, 677-684.

80) Norton GR, Majane HOI, Libhaber E, Maseko M, Makaula S *et al.* (2009). The relationship between blood pressure and left ventricular mass index depends on excess adiposity. *J Hypertens* **27**, 1873-1883.

81) Norton GR, Majane OHI, Redelinghuys M, Libhaber C, Maseko MJ *et al.* (2012). Brachial blood pressure-independent relations between radial late systolic shoulder-derived aortic pressures and target organ changes. *Hypertension* **59**, 885-892.

82) Omran AR (2005). The epidemiologic transition. A theory of the epidemiology of population change. *Milbank Q* **83**, 731-757.

83) Opie LH & Seedat YK (2005). Hypertension in Sub-Saharan African Populations. *Circulation* **112**, 3562-3568.

84) O'Brien E, Asmar R, Beilin L, Imai Y, Mallion JM *et al.* (2003). European Society of Hypertension Working Group on Blood Pressure Monitoring. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. *J Hypertens* **21**, 821-848.

85) O'Donnell CJ, Demissie S, Kimura M, Levy D, Gardner JP, White C *et al.* (2008). Leukocyte telomere length and carotid artery intimal medial thickness: The Framingham heart study. *Arterioscler Thromb Vasc Biol* **28**, 1165-1171.

86) Pablos-Mendez A, Mayeux R, Ngai C, Shea S & Berglund L (1997). Association of Apo E polymorphism with plasma lipid levels in a multiethnic elderly population. *Arterioscler Thromb Vasc Biol* **17**, 3534-3541.

87) Paolisso G, Tagliamonte MR, Galderisi M, Zito GA, Petrocelli A *et al.* (1999). Plasma leptin level is associated with myocardial wall thickness in hypertensive insulin-resistant men. *Hypertension* **34**, 1047-1052.

88) Paul TK, Chen W, Srinivasan SR, He J & Berenson GS (2011). Contrast of the impact of multiple cardiovascular risk factors on the femoral and carotid intima-media thickness in asymptomatic young adults: The Bogalusa Heart Study. *Atherosclerosis* **216**, 359-364.

89) Pignoli P, Tremoli E, Poli A, Oreste P, & Paoletti R (1986). Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation* **74**, 1399-1406.

90) Polak JF (2009). Carotid intima-media thickness an early marker of cardiovascular disease. *Ultrasound Quarterly* **25**, 55-61.

91) Poredos P (2004). Intima-media thickness: indicator of cardiovascular risk and measure of the extent of atherosclerosis. *Vasc Med* **9**, 46-54.

92) Predazzi IM, Martínez-Labarga C, Vecchione L, Mango R, Ciccacci C *et al.* (2010). Population differences in allele frequencies at the OLR1 locus may suggest geographic disparities in cardiovascular risk events. *Ann Hum Biol* **37**, 136-148.

93) Prentice AM & Jebb SA (2001). Beyond body mass index. *Obes Rev* **2**, 141-147.

94) Puoane T, Steyn K, Bradshaw D, Laubscher R, Fourie J, Lambert V *et al.* (2002). Obesity in South Africa: The South African Demographic and Health Survey. *Obesity Research* **10**, 1038-1048.

95) Raitakari OT, Juonala M, Kahonen M, Taittonen L, Laitinen T *et al.* (2003). Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: The Cardiovascular Risk in Young Finns Study. *JAMA* **290**, 2277-2283.

96) Rossouw JE (2002). Hormones, genetic factors, and gender differences in cardiovascular disease. *Cardiovascular Research* **53**, 550-557.

97) Salonen R & Salonen JT (1991). Determinants of carotid intima-media thickness: a population-based ultrasonography study in Eastern Finnish men. *Journal of Internal Medicine* 229, 225-231.

98) Sarmento PL, Plavnik FL, Zanella MT, Pinto PE, Miranda RB & Ajzen SA (2009).Association of carotid intima-media thickness and cardiovascular risk factors in women pre- and post-bariatric surgery. *Obes Surg* **19**, 339-344.

99) Schaefer EJ, Lamon-Fava S, Cohn SD, Schaefer MM, Ordovas JM *et al.* (1994). Effects of age, gender, and menopausal status on plasma low density lipoprotein cholesterol and apolipoprotein B levels in the Framingham Offspring Study. *J Lipid Res* **35**, 779-792.

100) Seidell JC, Oosterlee A, Deurenberg P, Hautvast JG & Ruijs JH (1988). Abdominal fat depots measured with computed tomography: effects of degree of obesity, sex, and age. *Eur J Clin Nutr* **42**, 805-815.

101) Sharma K, Blaha MJ, Blumenthal RS & Musunuru K (2009). Clinical and research applications of carotid intima-media thickness. *AM J Cardiol* **103**, 1316-1320.

102) Shiburi CP, Staessen JA, Maseko M, Wojciechowska W, Thijs L *et al.* (2006). Reference values for sphygmocor measurements in South Africans of African ancestry. *AM J Hypertens* **19**, 40-46.

103) Shipley MJ, Pocock SJ & Marmot MG (1991). Does plasma cholesterol concentration predict mortality from coronary heart disease in elderly people? 18 year follow up in Whitehall study. *BMJ* **303**, 89-92.

104) Siffert W, Fortser P, Jockel K, Mvere DA, Brinkmann B *et al.* (1999). Worl wide ethnic distribution of the G protein β3 subunit 825T allele and its association with obesity in Caucasian, Chinese, and Black African individuals. *J Am Soc Nephrol* **10**, 1921-1930.

105) Skilton MR, Serusclat A, Sethu A, Brun S, Bernard S *et al.*(2009). Noninvasive measurement of carotid extra-media thickness: Associations with cardiovascular risk factors and intima-media thickness. *J Am Coll Cardiol Img* **2**, 176-182.

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

107) Sliwa K, Damasceno A & Mayosi BM (2005). Epidemiology and etiology of cardiomyopathy in Africa. *Circulation* **112**, 3577-3583.

108) Soliman EZ, Ding J, Hsu F, Carr J, Polak JF *et al.* (2010). Association between carotid intima-media thickness and pericardial fat in the Multi-Ethnic Study of Atherosclerosis (MESA). *J Stroke Cerebrovasc Dis* **19**, 58-65.

109) Srinivasan SR, Frerichs RR, Webber LS & Berenson GS (1976). Serum lipoprotein profile in children from a biracial community: The Bogalusa Heart Study. *Circulation* **54**, 309-318.

110) Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB *et al.* (2008). Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: A consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force endorsed by The Society for Vascular Medicine. *J Am Soc Echocardiogr* **21**, 93-111.

111) Steinberg D, Parthasarathy S, Carew TE, Khoo JC & Witzum (1989). Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* **320**, 915-924.

112) Stephenson K, Tunstead J, Tsai A, Gordon R, Henderson S *et al.* (2003). Neointimal formation after endovascular arterial injury is markedly attenuated in db/db mice. *Arterioscler Thromb Vasc Biol* **23**, 2027-2033.

113) Stewart S, Wilkinson D, Hansen C, Vaghela V, Mvungi R, McMurray J *et al.* (2008).Predominance of heart failure in the Heart of Soweto Study cohort: emerging challenges for urban African communities. *Circulation* **118**, 2360-2367.

114) Steyn K, Gaziano TA, Bradshaw D, Laubscher R & Fourie J (2001). Hypertension in South African adults: results from the Demographic and Health Survey, 1998. *J Hypertens* **19**, 1717-1725.

115) Steyn K, Sliwa K, Hawken S, Commerford P, Onen C, Damasceno A *et al.* (2005). Risk factors associated with myocardial infarction in Africa: The INTERHEART Africa study. *Circulation* **112**, 3554-3561.

116) Steyn K & Fourie JM (2007). THE HEART AND STROKE FOUNDATION SOUTH AFRICA. Heart disease in SOUTH AFRICA: *MEDIA DATA DOCUMENT*. 1-29.

117) Stocker R & Keaney JF (2004). Role of oxidative modifications in atherosclerosis. *Physiol Rev* 8, 1381-1478.

118) Strong K, Mathers C & Bonita R (2007). Preventing stroke: saving lives around the world. *Lancet Neurol* **6**, 182-87.

119) Tan TY, Lu CH, Lin TK, Liou CW, Chuang YC & Schminke U (2009). Factors associated with gender difference in the intima-media thickness of the common carotid artery. *Clinical Radiology* **64**, 1097-1103.

120) Taylor AJ, Kent SM, Falherty PJ, Coyle LC, Markwood TT *et al.* (2002). ARBITER: Arterial biology for the investigation of the treatment effects of reducing cholesterol. A randomized trial comparing the effects of atorvastatin and pravastatin on carotid intimal media thickness. *Circulation* **106**, 2055-2060.

121) Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel 111) final report (2002). *Circulation* **106**, 3143-3421.

122) Thom S (1997). Arterial structural modifications in hypertension. Effects of treatment. *Eur Heart J* **18**, E2-E4.

123) Tibazarwa K, Ntyintyane L, Sliwa K, Gerntholz T, Carrington M, Wilkinson D *et al.* (2009). A time bomb of cardiovascular risk factors in South Africa: Results from the heart of Soweto study "Heart Awareness Days". *ijcard* **132**, 233-239.

124) Touboul PJ, Prati P, Scarabin PY, Adrai V, Thibout E *et al.* (1992). Use of monitoring software to improve the measurement of carotid wall thickness by B-mode imaging. *J Hypertens* **Suppl 10**, 37- 41.

125) Touboul PJ, Hennerici MG, Meairs S ,Adams, Amarenco P *et al.* (2007). Mannheim Carotid Intima-Media Thickness Consensus (2004-2006). *Cerebrovasc Dis* **23**, 75-80.

126) Tzou WS, Douglas PM, Srinivasan SR, Bond MG, Tang R *et al.* (2007). Distribution and predictors of carotid intima-media thickness in young adults. *Prev Cardiol.* **10**,181-189.

127) Urbina EM, Srinivasan SR, Tang R, Bond MG, Kieltyka L *et al.* (2002). Impact of multiple coronary risk factors on the intima-media thickness of different segments of carotid artery in healthy young adults (The Bogalusa Heart Study). *Am J Cardiol* **90**, 953-958.

128) Urbina EM, Kimball TR, McCoy CE, Khoury PR, Daniels SR &Dolan LM (2009). Youth with obesity and obesity-related type 2 diabetes mellitus demonstrate abnormalities in carotid structure and function. *Circulation* **119**, 2913-2919.

129) Veller MG, Fisher CM, Nicolaides AN, Renton S, Geroulakos G *et al.* (1993). Measurement of the ultrasonic intima-media complex thickness in normal subjects. *J Vasc Surg* **17**, 719-725.

130) Watkins LO (1984). Epidemiology of coronary heart disease in black populations: Methodologic proposals. *Am Heart J* **108**, 635-640.

131) Watkins LO, Neaton JD& Kuller LH (1986). Racial differences in high-density lipoprotein cholesterol and coronary heart disease incidence in the

usual care group of the Multiple Risk Factor Intervention Trial. *Am J Cardiol* **57**,538-545.

132) Wiegman A, Hutten BA, de Groot E, Rodenburg J, Bakker HD *et al.* (2004). Efficacy and safety of statin therapy in children with familial hypercholesterolemia: A randomized controlled trial. *JAMA* **292**, 331-337.

133) Wilson PW & Grundy SM (2003). The metabolic syndrome: A practical guide to origins and treatment: Part II. *Circulation* **108**, 1537-1540.

134) Wittekoek ME, de Groot E, Prins MH, Trip MD, Buller HR *et al.* (1999). Differences in intima-media thickness in the carotid and femoral arteries in familial hypercholesterolemic heterozygotes with and without clinical manifestations of cardiovascular disease. *Atherosclerosis* **146**, 271-279.

135) Yusuf S, Reddy S, Ounpuu S & Anand S (2001). Global burden of cardiovascular diseases: Part II: variations in cardiovascular disease by specific ethnic groups and geographic regions and prevention strategies. *Circulation* **104**, 2855-2864.

Appendix 1

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Woodiwiss/Norton

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER MO70469**
PROJECT	Gene Candidates As Determinants of Blood Pressure and Intermediary Phenotypes In Pathogenesis of Hypertension in Black S Africans
INVESTIGATORS	Profs A/G Woodiwiss/Norton
DEPARTMENT	School of Physiology
DATE CONSIDERED	07.05.09
DECISION OF THE COMMITTEE*	Approved unconditionally (refer M020472)

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07.05.09 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Woodiwiss A Prof

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Woodiwiss/Norton et al

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M02-04-72

PROJECT Gene Candidates As Determinants of Blood Pressure And Intermediary Phenotypes In Pathogenesis of Hypertension In Black South Africans

INVESTIGATORS Prof's AJ/G et al Woodiwiss/Norton et al

DEPARTMENT School of Physiology, Wits Medical School

DATE CONSIDERED 02-04-26

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON-JONES
HREC (MEDICAL)
2007-05-09
JOHANNESBURG

DECISION OF THE COMMITTEE *

Approved unconditionally

This clearance is valid and within the Wits 5-year validity

DATE 02-05-14 **CHAIRMAN**.....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof AJ Woodiwiss
Dept of School of Physiology, Wits Medical School

Works2\lain0015\HumEth97.wdb\M 02-04-72
==========

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES