

The association between fibrinolysis markers and body composition in black adults in the North West Province of South Africa

P Eksteen

12826936

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Supervisor: Prof. M Pieters

Co-supervisor: Prof. HS Kruger

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**This mini-dissertation is dedicated to my father
Barnie Celliers,
with loving memories**

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ABSTRACT

INTRODUCTION

Plasminogen activator inhibitor type-1 (PAI-1) has a known relationship with obesity and more specifically with central obesity. Traditionally the physiological contribution of PAI-1 is seen as an indicator of fibrinolysis with increased PAI-1 levels contributing to decreased fibrinolysis. In more recent years, assays have been developed that not only uses proxy markers, such as PA-1, which is considered to be representative of fibrinolysis, but global assays that report on the global fibrinolytic potential of an individual, often reported as clot lysis time (CLT). Investigations into the relationship of CLT with obesity are scarce. Preliminary evidence shows that the relationship of CLT with obesity may differ from that of PAI-1 with obesity although in depth investigations in this regard are lacking. Therefore, the aim of this study was to investigate the association between fibrinolysis markers (PAI-1_{act} and CLT) and various markers of body composition in the South African Prospective Urban and Rural Epidemiology (PURE) data collected during 2010.

METHODS

Data collected in the PURE study in 2010 were cross-sectionally analysed. The participants (n = 1288) were apparently healthy black South-African men and women 35 years and older, residing in urban and rural settlements in the North-West Province. Experimental methods included anthropometric measurements such as height, weight, hip circumference, waist circumference, skinfolds (triceps, chest, abdominal, thigh and supra iliac skinfolds) and body composition measurements by means of air-displacement plethysmography and bioelectrical impedance analysis. Laboratory analysis of fibrinolysis markers, PAI-1_{act} and CLT were also performed.

MAIN FINDINGS

In men, similarities were seen regarding the relationship between PAI-1_{act} and body composition markers and the relationships observed between CLT and body composition markers. In contrast, in the women more and stronger associations were observed between CLT and body composition markers compared to that observed between PAI-1_{act} and body composition markers. CLT showed a linear relationship with body composition markers where PAI-1_{act} levels plateaued at higher body composition categories. Possible reasons for the observed differences may be related to differences in adipose tissue

distribution and sequence of accumulation between men and women. PAI-1 is associated with visceral adipose tissue (VAT) where high amounts of stromal cells are found. In men preferential accumulation of VAT may explain similarities in the relationship of PAI-1_{act} with body composition and that of CLT with body composition. Proportionally less VAT, but more subcutaneous adipose tissue in women may explain the observed increase in CLT compared to PAI-1_{act} levels that plateaued over body composition tertiles and categories.

CONCLUSION

PAI-1_{act} has a stronger association with central obesity while CLT has a stronger association with total body fat. In women PAI-1_{act} and CLT showed different associations with body composition markers, whereas associations of PAI-1_{act} and CLT with body composition were similar in men. PAI-1_{act} is strongly influenced by type of body fat accumulation whereas CLT is associated with obesity independent of type and sequence of body fat accumulation. Significant associations observed between CLT and body composition variables are, therefore, at least in part, independent of PAI-1_{act}. Additional factors such as, thrombin activatable fibrinolysis inhibitor (TAFI), α -2-antiplasmin, plasminogen, prothrombin and fibrin clot structure that influence CLT and are also related to obesity may additionally contribute to the link between CLT and obesity.

KEY WORDS: markers of fibrinolysis, PAI-1_{act}, CLT, body composition, black South African adults

OPSOMMING

INLEIDING

Plasminogeen aktiveerder inhibeerder tipe-1 (PAI-1) het 'n bekende verband met obesiteit en meer spesifiek met sentrale obesiteit. Tradisioneel is die fisiologiese bydrae van PAI-1 as 'n indikator van fibrinolise gesien, met die gevolg dat verhoogde PAI-1 vlakke bydra tot verlaagde fibrinolise. In die afgelope paar jaar is toetse ontwikkel wat nie net merkers, soos PAI-1, wat verteenwoordigend van fibrinolise is, gebruik nie, maar globale toetse wat die globale fibrinolitiese potensiaal van 'n individu bepaal. Hierdie potensiaal word gewoonlik gerapporteer as klontlisetyd (KLT). Studies oor die verhouding tussen KLT en obesiteit is skaars. Voorlopige bewyse dui daarop dat die verhouding tussen KLT en obesiteit mag verskil van die verhouding tussen PAI-1 en obesiteit ten spyte daarvan dat in-diepte studies aangaande hierdie verband skaars is. Daarom was die doel van hierdie studie om die verwantskap tussen merkers van fibrinolise (PAI-1_{akt} en KLT) en verskeie merkers van liggaamsamestelling in die Suid Afrikaanse *Prospective Urban and Rural Epidemiology* (PURE) data wat in 2010 versamel is, te ondersoek.

METODES

Data wat in die PURE studie in 2010 versamel is, is in 'n dwarsdeursnit studie-ontwerp geanaliseer. Die proefpersone (n = 1288) was oënskynlike gesonde swart Suid-Afrikaanse mans en vrouens van 35 jaar en ouer, wat in landelike en stedelike nedersettings woon. Eksperimentele metodes het ingesluit: antropometriese metings soos lengte, gewig, heupomtrek, middelomtrek, velvoue (trisepts-, pektorale-, abdomen-, dy- en supra-iliakvelvou) asook liggaamsamestellingmetings deur middel van lugverplasingspletismografie en bioelektriese-impedans analise. Laboratoriumontleding van merkers van fibrinolise, PAI-1_{akt} en KLT is ook uitgevoer.

HOOF BEVINDINGE

In die mans het die verwantskap tussen PAI-1_{akt} en merkers van liggaamsamestelling en die verwantskap tussen KLT en merkers van liggaamsamestelling baie ooreengestem. In kontras hiermee was daar in vrouens meer en sterker verwantskappe tussen KLT en merkers van liggaamsamestelling as tussen PAI-1_{akt} en merkers van liggaamsamestelling. KLT het 'n reglynige verband met merkers van liggaamsamestelling getoon terwyl PAI-1_{akt} vlakke afgeplat het by die hoër liggaamsamestelling kategorieë. Moontlike redes vir

hierdie verskille wat waargeneem is, mag verband hou met verskille in die verspreiding en volgorde van neerlegging van vetweefsel tussen mans en vrouens. PAI-1 word spesifiek geassosieer met viserale vetweefsel (VWV) wat groot hoeveelhede stromale selle bevat. Die feit dat mans by voorkeur VWV akkumuleer, mag moontlik die ooreenkomste in die verhouding tussen PAI-1_{akt} en merkers van liggaamsamestelling en dié van KLT en merkers van liggaamsamestelling verklaar. Proporsioneel minder VWV maar meer subkutane vetweefselneerlegging in vrouens mag moontlik die toename in KLT verklaar in teenstelling met PAI-1_{akt} vlakke wat afplat oor liggaamsamestelling tertiele en kategorieë.

GEVOLGTREKKING

PAI-1_{akt} het 'n sterker verwantskap met sentrale obesiteit terwyl KLT 'n sterker verwantskap met totale liggaamsvet het. In die vrouens toon PAI-1_{akt} en KLT verskillende verwantskappe met merkers van liggaamsamestelling, terwyl die verwantskap van PAI-1_{akt} en KLT met merkers van liggaamsamestelling ooreenstem in die mans. PAI-1_{akt} word sterk beïnvloed deur die tipe liggaamsvet wat geakkumuleer word, terwyl KLT se assosiasie met obesiteit onafhanklik is van die tipe en patroon van toename in vetweefsel. Die betekenisvolle verwantskappe wat tussen KLT en merkers van liggaamsamestelling waargeneem is, is in 'n mate onafhanklik van PAI-1_{akt}. Addisionele faktore soos trombin, trombin-geaktiveerde fibrinolise inhibeerder (TAFI), α -2-antiplasmin, plasminogeen, protrombin en fibrienklontstruktuur, wat KLT beïnvloed maar wat ook 'n verband met obesiteit het, mag 'n bydraende rol speel om die verhouding tussen KLT en obesiteit te verklaar.

SLEUTELTERME: merkers van fibrinolise, PAI-1_{akt}, KLT, liggaamsamestelling, swart Suid-Afrikaanse volwassenes

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LIST OF ABBREVIATIONS

a	Activated
ADP	Air-displacement plethysmography
AIDS	Acquired immune deficiency syndrome
ANCOVA	Analysis of co-variance
ANOVA	Analysis of variance
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CEN	Centre of Excellence of Nutrition
CHD	Coronary heart disease
CLoFAL	Clot formation and lysis
CLT	Clot lysis time
cm	Centimetre
CRP	C-reactive protein
CV	Cardiovascular
CVD	Cardiovascular disease
DWBCLT	Dilute whole-blood clot lysis time
ECLT	Euglobulin clot lysis time
ETP	Endogenous thrombin generation potential
F	Factor
F1	Fragment 1
F2	Fragment 2
g	Gram
g/L	Gram per litre
GFA	Global fibrinolytic assay
GFC	Global fibrinolytic capacity
Glu	Glutamic acid
HART	Hypertension in Africa Research Team
HDL-C	High-density lipoprotein cholesterol
HIV	Human immunodeficiency virus
IQR	Interquartile range
IU	International Unit
IU/ml	International units per millilitre

kDa	Kilodalton
kg	Kilogram
kg/m ²	Weight by height squared
KW	Kruskall-Wallis
LDL-C	Low-density lipoprotein cholesterol
Lys	Lysine
m	Metre
min	Minutes
ml	Millilitre
mM	Milli molar
mm	Millimetre
mmol/L	millimol per litre
n	Population / sample size
NCD	Non-communicable disease
ng/ml	Nano gram per millilitre
NIH	National Institute of Health
nm	Nanometre
NWU	North-West University
OCP	Overall coagulation potential
OFP	Overall fibrinolysis potential
OHP	Overall haemostatic potential
PAI	Physical activity index
PAI-1	Plasminogen activator inhibitor type-1
PAI-1 _{act}	PAI-1 activity
PAI-1 _{ag}	PAI-1 antigen
PAI-2	Plasminogen activator inhibitor type-2
PAI-3	Plasminogen activator inhibitor type-3
PAR	Plasminogen activator reagent
pM	Pico molar
PURE	Prospective Urban and Rural Epidemiology
p-value	Statistical significance test
r	Correlation coefficient
SANHANES	South African National Health and Nutrition Examination Survey
SAT	Subcutaneous adipose tissue
TAFI	Thrombin-activatable fibrinolysis inhibitor

TC	Total cholesterol
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TGF- β	Transforming growth factor- β
THUSA	Transition and Health during Urbanisation in South Africa
TSF	Triceps skinfold
TNF- α	Tumor necrosis factor- α
t-PA	Tissue-type plasminogen activator
UK	United Kingdom
u-PA	Urokinase-type plasminogen activator
USA	United States of America
VAT	Visceral adipose tissue
VLDL	Very low-density lipoprotein
vs	Versus
vWF	Von Willebrand factor
WC	Waist circumference
WHO	World Health Organisation
WHR	Waist: hip ratio

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

As a degenerative and chronic disease of lifestyle, cardiovascular disease (CVD) is well documented as a global problem and contributes significantly to the global burden of disease. With CVD remaining the single leading cause of death, projected deaths due to CVD will increase from an estimated 17.3 million in 2008 to 25 million in 2030 (WHO, 2012a; WHO, 2012b).

CVD leads to events that include myocardial infarction, stroke and venous thrombosis. The underlying aetiology of a CVD event is an atherothrombotic process (Collen & Lijnen, 1991). Factors that play a crucial role in this process are endothelial dysfunction, inflammation and increased levels of proteins involved in coagulation. Atherosclerotic plaque development takes place in stages over time, but with advanced age it becomes unstable and eventually ruptures, exposing a prothrombotic core. Both platelets and coagulation factors are activated by this, resulting in the formation of a thrombus, which may occlude a blood vessel. Occlusion of a blood vessel results in ischaemic symptoms due to loss of blood flow to vital organs (Ajjan & Ariëns, 2009). Fibrinolysis or the breakdown of a thrombus is a protective mechanism against thrombotic occlusion. Thus the ability of the body to lyse a thrombus quickly is very important (Gorog, 2010).

In the fibrinolytic pathway the inactive pro-enzyme, plasminogen, is converted to active plasmin by thrombin. Plasmin is responsible for the degradation of the cross-linked fibrin into soluble degradation products with the assistance of tissue-type and urokinase type plasminogen activators. Tissue-type plasminogen activator (tPA) is mainly responsible for the dissolution of fibrin formed in the circulation. Inhibition of the fibrinolytic system is either by antagonising plasmin through α -2-antiplasmin or by specific plasminogen activator inhibitors (PAIs). Of the three types of PAI, PAI type-1 (PAI-1) is the primary physiological inhibitor of plasminogen activation in the blood (Gorog, 2010; Rijken & Lijnen, 2009).

It is possible to measure the ability of the body to lyse clots in different ways. As already mentioned, plasminogen, PAI-1 and tPA are all proteins involved in the fibrinolytic pathway. The activity and concentration of these three proteins can for example be measured individually, acting as proxy markers for fibrinolysis. Alternatively, global fibrinolytic assays (GFA) can be used to measure the global ability of the blood or plasma to lyse clots. These assays, often reported as clot-lysis time (CLT), give an indication of the speed with which the body can lyse clots. However, the GFA does not provide information on the individual components of the fibrinolytic system (Lisman *et al.*, 2001).

Hypofibrinolysis or reduced plasma fibrinolytic potential (i.e. longer CLT) has been shown, in various studies conducted in Caucasian populations, to be associated with CVD events. Hypofibrinolysis has been shown to increase the risk of a first myocardial infarction in young men (Meltzer *et al.*, 2009) and to increase the relative risk of arterial thrombosis also in young subjects (Guimarães *et al.*, 2009). Lisman *et al.* (2005) and Meltzer *et al.* (2008) found that subjects with reduced fibrinolytic potential have an increased risk for the development of venous thrombosis.

In terms of CVD risk factors, Meltzer *et al.* (2009) have shown in Caucasian subjects that CLT increased with increasing body mass index (BMI), increased total cholesterol (TC), triglycerides and C-reactive protein (CRP), and decreased with increasing high-density lipoprotein cholesterol (HDL-C). In another study in Caucasians, a linear trend was also observed between CLT and BMI. In the same study, subjects with diabetes also exhibited higher CLT's than controls without diabetes (Guimarães *et al.*, 2009). The levels of PAI-1 have also been related to adverse cardiovascular outcomes in both healthy individuals and cardiac patients as reported by several studies and summarised by Gorog (2010). In obesity, a well-known risk factor for CVD, PAI-1 is the protein of the haemostatic system that is found to be the most disordered (Mutch *et al.*, 2001). Elevated PAI-1 levels have been shown to be associated with obesity, but more specifically with central obesity (Hoekstra *et al.*, 2004; Juhan-Vague *et al.*, 2002; Sartori *et al.*, 2001).

In South Africa the black African population is experiencing an increased prevalence of CVD (Sliwa *et al.*, 2008; Steyn *et al.*, 2005), probably attributable to a significant

health transition due to urbanisation of the black South African population. This urbanisation is accompanied by decreased physical activity and changes to a Westernised lifestyle and diet (Vorster *et al.*, 2000). The risk factors for CVD have been shown to increase with urbanisation, as demonstrated by two epidemiological studies in black South Africans. One such study is the Transition and Health during Urbanisation in South Africa (THUSA) study and the other one is the Prospective Urban and Rural Epidemiology (PURE) study. From the THUSA study it was reported that in both blood pressure and total serum cholesterol levels in men and women, smoking prevalence in men and BMI in women had increased with urbanisation (Vorster, 2002). Pieters *et al.* (2011) found that in the PURE population several factors associated with CVD were significantly higher in the urban subjects compared to the rural subjects. These factors include fasting plasma glucose, triglyceride concentrations, waist circumference (WC), BMI, blood pressure and PAI-1_{act}.

Until recently no information was available regarding the relationship between fibrinolytic potential and CVD risk factors in Africans. In a recent investigation by De Lange *et al.* (2012), it was found that the main predictors of CLT in black South Africans from the PURE population were PAI-1_{act} (28%), BMI (8%), alcohol consumption (4%) and HbA1c (2%). In this study, there was a stronger correlation between CLT and BMI (an indicator of total overall obesity) than with WC (an indicator of visceral fat / central obesity). This association between BMI and CLT remained after adjustment for PAI-1_{act}. This is very noteworthy since in the same population, PAI-1_{act} (a major predictor of CLT) had a significantly stronger correlation with WC than BMI (Pieters *et al.*, 2010). PAI-1 is synthesised and secreted by human adipose tissue (Dellas & Loskutoff, 2005). Compared to subcutaneous fat, PAI-1 secretion is more pronounced in visceral fat, and the stromal cells within the adipose tissue have been identified as an important source of PAI-1 (Bastelica *et al.*, 2002; Cigolini *et al.*, 1999). More pronounced PAI-1 production from excess visceral fat, as seen in central obesity, may therefore explain the stronger correlation of PAI-1 with central obesity than with total obesity. Therefore the question that now arises is to what extent the relationship between CLT and obesity can be explained by PAI-1, and whether there are other independent factors or mechanisms that are involved in this relationship?

Based on the differences in the relationship between CLT (marker of plasma fibrinolytic potential) and body composition and PAI-1 and body composition in the PURE study population in 2005, and since the follow-up South African PURE data (2010) contains more detailed body composition measurements as well as information on physical activity, we decided to investigate the association between fibrinolysis markers (PAI-1_{act} and CLT) and body composition and between fibrinolysis markers (PAI-1_{act} and CLT) and physical activity in more detail.

1.2 AIM AND OBJECTIVES

The main aim of this study was to investigate the association between fibrinolysis markers (PAI-1_{act} and CLT) and various markers of body composition in the South African PURE data collected during 2010.

In order to reach the above mentioned aim, the data of the PURE population (n = 1288), which was collected in 2010, was used in this present cross-sectional study. The specific objectives were:

1. To measure PAI-1_{act} in the South African PURE population (2010) (n = 1288).
2. To measure CLT as a marker of global plasma fibrinolytic potential (Lisman *et al.*, 2005), in the South African PURE population (2010) (n = 1288).
3. To investigate the relationship between:
 - 3.1 PAI-1_{act} and markers of body composition including BMI, WC, waist: height ratio, waist: hip ratio (WHR) and skinfolds (triceps, chest, abdominal, thigh and supra iliac skinfolds) in the total study population (n = 1288);
 - 3.2 PAI-1_{act} and body fat percentage, fat mass and fat-free mass in an urban sub-group of the study population (n = 144) by means of air-displacement plethysmography (ADP) using a *BodPod®; and in the rural group (n = 697) by means of bioelectrical impedance analysis (BIA) using a *Tanita Ironman™.

4. To investigate the relationship between:
 - 4.1 plasma fibrinolytic potential (expressed as clot lysis time (CLT)) and markers of body composition including BMI, WC, waist: height ratio, WHR and skinfolds (triceps, chest, abdominal, thigh and supra iliac skinfolds) in the total study population (n = 1288);
 - 4.2 global plasma fibrinolytic potential and body fat percentage, fat mass and fat-free mass in an urban sub-group of the study population (n = 144) by means of ADP using a *BodPod[®]; and in the rural group of the study population (n = 697) by means of BIA using a *Tanita Ironman[™].
5. To compare the relationship between PAI-1_{act} and the abovementioned markers of body composition with the relationship between global plasma fibrinolytic potential and the abovementioned markers of body composition.
6. To investigate the relationship between PAI-1_{act}, CLT and physical activity in the study population (n = 1288).

*The BodPod[®] could only be used on an urban subgroup of 144 participants due to practical reasons. After 144 urban participants were measured the instrument broke down and it was replaced with the Tanita Ironman[™] that was sent from Canada. Unfortunately by the time it reached South Africa it was too late to measure the rest of the urban participants. Another reason for the BodPod[®] being used only in the urban group was that this is a very sensitive instrument and that it could not be transported over long distances to the rural areas. Rural-urban comparisons were not performed for these body composition variables due to the two different measurement techniques used.

1.3 RESEARCH TEAM

The PURE study was conducted by a multi-disciplinary team. The list of names below state only the individuals who were directly involved in this research project and laboratory analysis.

Title	Affiliation	Role in the study
Prof. M Pieters	Centre of Excellence of Nutrition (CEN), North-West University (NWU), Potchefstroom Campus	Supervisor of the MSc dissertation. Guidance regarding protocol, writing of the literature review, statistical analysis, interpretation of results and writing up of the data.
Prof. HS Kruger	CEN, NWU, Potchefstroom Campus	Co-supervisor of the MSc dissertation. Guidance regarding protocol, writing of the literature review, statistical analysis, interpretation of results and writing up of the data.
Dr. Z De Lange	CEN, NWU, Potchefstroom Campus	Laboratory analysis of CLT.
Ms. A Jobse	CEN, NWU, Potchefstroom Campus	Laboratory analysis of PAI-1 _{act} .
Ms. E Rossouw	CEN, NWU, Potchefstroom Campus	Laboratory analysis of PAI-1 _{act} .
Ms. P Eksteen	CEN, NWU, Potchefstroom Campus	Part-time MSc student. Writing of the protocol, assisted with laboratory analysis of haemostatic variables, writing up of the literature review and methodology, statistical analysis, interpretation of results and writing up of data.

1.4 STRUCTURE OF THIS DISSERTATION

This mini-dissertation in chapter format contains 5 chapters. The mini-dissertation was language edited and technically edited in the Harvard style as required by the North-West University. Chapter 1, which is the introductory chapter, is followed by Chapter 2, the literature review. This literature review starts with a brief overview of

the haemostatic and fibrinolytic systems. This is followed by a discussion of PAI-1 and its role in CVD as well as the association between PAI-1 and body composition. The different methods for the measurement of global plasma fibrinolytic potential are then shortly reviewed, where after the association between fibrinolytic potential and body composition is discussed. The literature review ends with a short discussion about the link between physical activity and fibrinolysis.

The methodology of this study is discussed in Chapter 3. This chapter provides an explanation of the ethical approval that was obtained, the design of the study, the recruitment method and characteristics of the participants, followed by a description of the study procedure and data collection tools. The experimental methods including blood pressure measurements, body composition measures, blood sampling and biochemical analyses are then explained. Laboratory methods to determine PAI-1_{act} and plasma fibrinolytic potential are explained in detail. Lastly the methods used to analyse the data statistically are included.

The results of this study are presented in Chapter 4. The basic characteristics of the South African PURE population in 2010 are presented first. This is followed by a presentation of the correlations of PAI-1_{act} level and CLT, as markers of fibrinolysis, with different body composition and physical activity variables for men and women separately. This is followed by a comparison of PAI-1_{act} level and CLT, across tertiles and categories of different body composition variables. The differences in the association between PAI-1_{act} and CLT with the body composition variables are also investigated. Lastly gender differences were also identified and highlighted.

The results of this study in the context of the current literature are then discussed in Chapter 5. Throughout this chapter possible reasons or explanations for the results obtained are given. This chapter briefly describes the anthropometric and fibrinolytic profiles of the men and women in the study population. This is followed by a discussion of the associations of PAI-1_{act} and CLT with different body composition and physical activity variables. Differences and comparisons in PAI-1_{act} and CLT between tertiles and categories of body composition variables in men and women are then discussed. This chapter ends with a short discussion about the limitations of the study and a conclusion.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

The high and growing burden of non-communicable diseases (NCDs) is well documented as a global problem (Fernstrom *et al.*, 2012; WHO, 2011a; Yusuf *et al.*, 2001). In 2008, it was estimated that of the 57 million global deaths in that year, 63% (36 million) were due to NCDs (Fernstrom *et al.*, 2012; WHO, 2011b; WHO, 2011c). Due to population growth and increased longevity, the number of deaths caused by NCDs continues to rise (WHO, 2012a). NCDs include cardiovascular disease (CVD), some types of cancer, type 2 diabetes and chronic respiratory disease with CVD causing the largest proportion of NCD deaths (48%) (Fernstrom *et al.*, 2012; WHO, 2009).

CVD remains the number one cause of death globally and continues to contribute significantly to the global burden of disease (WHO, 2012a). Already in 1997, it was predicted that the proportionate share of the global burden of disease due to CVD would rise from 11.1% in 1990 to 14.7% in 2020 (Murray & Lopez, 1997). Estimated deaths globally from CVD in 2008 were 17.3 million, of which an estimated 7.3 million were due to coronary heart disease (CHD) and 6.2 million due to stroke or another form of cerebrovascular disease. It is projected that deaths due to CVD will increase to 25 million in 2030 and that it will remain the single leading cause of death (WHO, 2012a; WHO, 2012b).

CVD is not only prevalent in developed countries. In 2008, around 80% of all NCD deaths (29 million) occurred in low-income and middle-income countries, occurring almost equally in men and women (WHO, 2012a). For middle-income countries, on the list of the 10 leading causes of death as projected for 2030 globally, cerebrovascular disease ranks first and ischaemic heart disease second (Mathers & Loncar, 2006).

Apart from CVD, sub-Saharan African countries are additionally experiencing a dual burden of both infectious and chronic diseases (Dalal *et al.*, 2011; Mathers & Loncar,

2006). Traditional diseases such as malaria and tuberculosis are joined by HIV/AIDS and occur concurrently with these NCDs (Bradshaw *et al.*, 2003a). The increased prevalence of NCDs and subsequently CVD in sub-Saharan Africa, including South Africa, has contributed to the disease emerging as a major cause of morbidity and mortality (Bloomfield *et al.*, 2011; BeLue *et al.*, 2009; Akinboboye *et al.*, 2003).

Seen from a historical perspective, the prevalence of CVD was rare in black South Africans up to the early to late 1990s (Seedat *et al.*, 1992; Seftel, 1978). However, as the rates of CVD are increasing in the developing world (Deeg *et al.*, 2008), an increase in the prevalence of CVD morbidity and mortality in the black South African population is also being seen (Sliwa *et al.*, 2008; Steyn *et al.*, 2005). This increase in prevalence is seen especially in urban areas (Stewart *et al.*, 2011; Sliwa *et al.*, 2008).

In South Africa coronary artery disease, hypertensive heart disease and stroke already account for more than a third of deaths in people older than 65 years (Bradshaw *et al.*, 2003b). More recent statistics indicate that CVD ranked fifth during 2007, 2008 and 2009 in the ten leading natural causes of death. CVD accounted for at least 4.3% of all deaths in 2007; 4.4% in 2008; and 4.6% in 2009 (Statistics South Africa, 2011). Looking at trends in the causes of death over a ten-year period (1997-2007), Bradshaw *et al.* (2012) reported an increase in the proportion of deaths due to CVD.

The increased prevalence of CVD in black South Africans is directly related to a rise in the prevalence of cardiovascular (CV) risk factors (Hernández *et al.*, 2012; Dalal *et al.*, 2011; Alberts *et al.*, 2005; Akinboboye *et al.*, 2003). Both the increased prevalence of CVD and CV risk factors are probably attributable to a significant health transition due to migration and modernisation of black South Africans from rural areas to urban communities. This urbanisation is accompanied by decreased physical activity and changes to a Westernised lifestyle and diet (Vorster *et al.*, 2000). The risk factors for CVD have been shown to increase with urbanisation and the lifestyle changes associated with it (Pieters & Vorster, 2008; Akinboboye *et al.*, 2003; Vorster, 2002).

Two well-documented epidemiological studies in black South Africans demonstrate the effect of urbanisation on the prevalence of CV risk factors. One such study is the Transition and Health during Urbanisation in South Africa (THUSA) study (Vorster *et al.*, 2007; Vorster, 2002; Vorster *et al.*, 2000) and the other one is the Prospective Urban and Rural Epidemiology (PURE) study (Teo *et al.*, 2009). The CV risk factors that have been shown to be affected by urbanisation in South Africa were increases in obesity, smoking, hypertension, serum lipids and fibrinogen (Pieters *et al.*, 2011; Vorster, 2002).

There are several well-known potentially modifiable risk factors for CVD. These risk factors include a history of diabetes mellitus and/or hypertension, obesity, an increased body mass index (BMI), hypercoagulability, hypertriglyceridemia, dyslipidaemia, cigarette smoking, physical inactivity and certain dietary patterns (Amira *et al.*, 2006; Yusuf *et al.*, 2004; Akinboboye *et al.*, 2003). The burden of CVD morbidity and mortality is linked to the modifiable CVD risk factors. There is substantial evidence of differences in the incidence and prevalence of these risk factors by race and/or ethnicity (Kurian & Cardarelli, 2007; Yusuf *et al.*, 2001). Until more recently, data on the profile of CVD and its risk factors in the black African population were scarce (Nethononda *et al.*, 2004; Budoff *et al.*, 2002).

There is specifically only limited information available regarding the haemostatic profile of black South Africans (Pieters & Vorster, 2008), while studies in Caucasians have revealed several proteins involved in blood clot formation and breakdown (fibrinolysis) contribute significantly to CVD development (Fibrinogen Studies Collaboration, 2005; Fibrinogen Studies Collaboration, 2004; Salomaa *et al.*, 1995). Both hypercoagulability and hypofibrinolysis have independently been shown to be related to CVD (Kannel, 2005; Lowe *et al.*, 2002). Increased clot formation and/or decreased lysis will result in clots remaining in the vasculature for a longer time period, increasing the risk of occlusion of blood vessels or embolism formation (Gale, 2010; Ogedegbe, 2002). Previous work from the PURE 2005 study, investigating the fibrinolytic system in black South Africans, revealed that plasminogen activator inhibitor type-1 (PAI-1), an important inhibitor of clot lysis, associated differently with markers of body composition compared with the association of clot lysis time (CLT) with markers of body composition (De Lange *et al.*, 2012). Therefore, the aim of this

study is to investigate these associations further, especially since the PURE 2010 data set contains more detailed body composition data. Of the known CV risk factors, this mini-dissertation is focused specifically on the relationship between body composition and fibrinolytic components as CVD risk factors. A brief overview of the haemostatic and fibrinolytic systems will now be given. This will be followed by a discussion on the association between PAI-1 and body composition and the association between global fibrinolytic potential and body composition. The literature review will end with a short discussion on the link between fibrinolysis and physical activity.

2.2 THE HAEMOSTASIS PROCESS

2.2.1 Overview of haemostasis

The underlying aetiology of a CVD event is an atherothrombotic process involving plaque formation (Collen & Lijnen, 1991). Factors that play a crucial role in this process are endothelial dysfunction, inflammation and increased levels of proteins involved in coagulation. Atherosclerotic plaque development takes place in stages over time, but with advanced age it becomes unstable and eventually ruptures, exposing a prothrombotic core (Ajjan & Ariëns, 2009). Both platelets and coagulation factors are activated by this, resulting in the formation of a thrombus, which may occlude a blood vessel. Occlusion of a blood vessel results in ischaemic symptoms due to loss of blood flow to vital organs (Ajjan & Ariëns, 2009). Fibrinolysis of a thrombus is a protective mechanism against arterial thrombotic occlusion. Thus, the ability of the body to lyse a thrombus quickly is very important (Gorog, 2010). Pearson *et al.* (1997) developed a model suggesting an association between haemostatic factors and CVD. Figure 1 is a presentation of this model. A short overview of the coagulation and fibrinolysis processes will now be given.

2.2.2 Coagulation

The process of coagulation is a cascade consisting of a three overlapping but consecutive steps: the initiation, amplification and propagation phases (Gale, 2010;

Ajjan & Grant, 2006; Hoffman & Monroe, 2001). In essence, when the coagulation cascade is activated, thrombin is generated, which in turn, converts fibrinogen to fibrin (Cesarman-Maus & Hajjar, 2005).

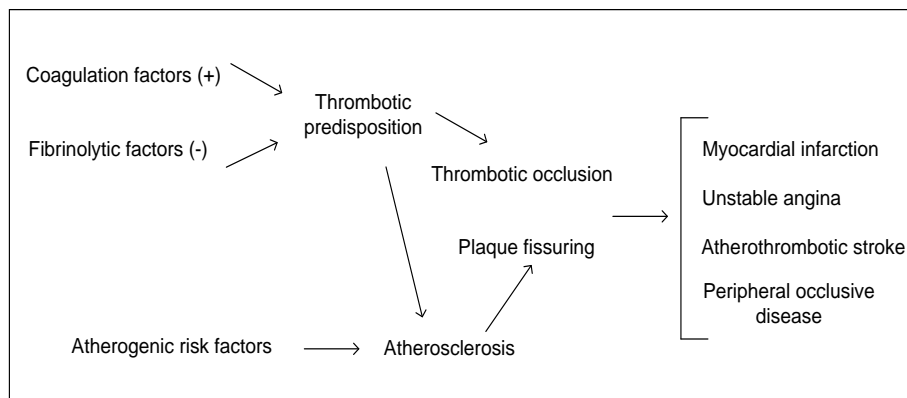


Figure 2.1: A model for the causal association of haemostatic factors and CVD. Coagulation and fibrinolytic factors can act either by promoting atherosclerosis or by causing thrombotic occlusion on a plaque fissure (Pearson *et al.*, 1997).

2.2.2.1 Initiation phase

Damage to the blood vessel wall and contact of plasma with tissue factor (TF) bearing cells initiates coagulation. The factor VII (FVII)/TF complex is formed when circulating FVII binds to TF found on the surface of a TF bearing cell. The activated FVII/TF complexes now activate plasma FIX and FX and in turn activated FX (FXa) activates factor V by binding to it. Both FXa and FVa generate a small amount of thrombin by the cleavage of prothrombin. The generation of thrombin partially activates platelets which adhere to collagen fibres that are exposed to blood (Hoffman & Monroe, 2007; Ajjan & Grant, 2006; Monroe & Hoffman, 2006; Briede *et al.*, 2001).

FXa is localised to the cell surface but any FXa that dissociates from the cell surface is quickly inhibited by inhibitors such as tissue factor pathway inhibitor (TFPI) and anti-thrombin (Monroe & Hoffman, 2006). Antithrombin inhibits FIXa at a much slower rate compared to the rate it inhibits FXa. FIXa is also not inhibited by TFPI. Thus compared to FXa, FIXa can move away from the TF bearing cell to nearby platelets. FIXa activate FX directly on the platelet surface by means of its interaction with its cofactor FVIIIa (Hoffman & Monroe, 2007; Monroe & Hoffman, 2006).

2.2.2.2 Amplification phase

In the second phase of the coagulation cascade, the pro-coagulant signal of thrombin being generated on the TF bearing cell is amplified (Hoffman & Monroe, 2007). Addition of more thrombin to collagen-bound platelets activates the platelets fully. Other factors that are also activated are FV, FVIII and FXI (Monroe & Hoffman, 2006; Frederick *et al.*, 2005). Adhesion of platelets to the damaged endothelium is accomplished through von Willebrand factor (vWF) that binds factor VIII through the formation of non-covalent complexes to form the vWF/FVIII complexes (Hoffman & Monroe, 2007; Ajjan & Grant, 2006). After binding of the vWF/FVIII complexes to platelets, FVIII is cleaved from vWF. This cleavage is accomplished by thrombin with the purpose to activate FVIII and to generate more thrombin (Frederick *et al.*, 2005; Hoffman & Monroe, 2001). Full activation of platelets and coagulation cofactors needs enough thrombin to be generated in the amplification phase. The amount of thrombin generated is enough to strengthen the initial pro-coagulant signal and to activate platelets. This sets the stage for generation of large amounts of thrombin (Hoffman & Monroe, 2007; Monroe & Hoffman, 2006).

2.2.2.3 Propagation phase

In this phase of the coagulation cascade, enough thrombin is generated on platelet surfaces for effective haemostasis. The fully activated platelets need now to change shape in the propagation phase. Change in the shape of the platelets is necessary for the following aspects in this part of the coagulation cascade: the binding of FIXa to FVIIIa on the platelet surface, additional supply of FIXa by platelet-bound FXIa, and providing the platelet surface with FXa from the FIXa/VIIIa complex. In addition, as mentioned previously, FXa is localized to the cell surface by anti-thrombin and TFPI. FXa needs to form a complex with FVa on the platelet surface. All these steps need to take place in order to produce enough thrombin to activate fibrinogen (Hoffman & Monroe, 2007; Monroe & Hoffman, 2006).

One of thrombin's functions is to facilitate the formation of fibrin from fibrinogen. Fibrinogen is a 340-kDa glycoprotein and contains two symmetrical sets of three polypeptide chains ($A\alpha$, $B\beta$ and γ). These polypeptide chains are held together with

disulfide bonds (Ajjan & Grant, 2006). Fibrinogen has a half-life of 3 – 4 days. Normally it is present in plasma concentrations of 2 - 3 g/L (Cook & Ubben, 1990; Standeven *et al.*, 2005).

Binding of thrombin to fibrinogen catalyses the cleavage of fibrinopeptides A and B from the A α - and B β -chains by means of hydrolysis (Ariens *et al.*, 2002). This converts the molecules to fibrin monomers and the monomers assemble into two-stranded protofibrils, which aggregate to form fibrin fibres. The fibrin fibres branch into a three-dimensional network trapping red blood cells, white blood cells and platelets to form a blood clot. Thrombin and calcium further stabilize the structure of the clot. Through a transglutaminase reaction, calcium activates FXIII to cross-link the fibrin fibres for stability (Ariens *et al.*, 2002; Colman, 2000:11; Ryan *et al.*, 1999). Fibrinolysis, the process through which clots can be broken down, will be discussed next.

2.2.3 Fibrinolysis

The purpose of fibrinolysis is the dissolution of blood clots during the process of wound healing and prevention of blood clots forming in healthy blood vessels. Fibrinolysis takes place on the surface of fibres of existing clots. Fibrin formation triggers the activation of fibrinolysis (Gale, 2010; Medved & Nieuwenhuizen, 2003). In essence, fibrinolysis is the process where plasmin breaks down a cross-linked fibrin clot into fibrinogen degradation products.

The fibrinolytic system consists of three pro-enzymes (zymogens) in the blood, plasminogen, tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) as well as several inhibitors (Gale, 2010). The zymogen plasminogen is a single-chain glycoprotein and produced in the liver (Gale, 2010). Plasminogen circulates in the form of amino-terminal glutamic acid (Glu) plasminogen. Glu-plasminogen is converted by means of proteolysis into several modified forms known as amino-terminal lysine (Lys) plasminogen. Modified Lys-plasminogen is activated 10-20 times more rapidly than Glu-plasminogen (Cesarman-Maus & Hajjar, 2005).

Two plasminogen activators exist in the circulation to convert plasminogen into plasmin. Of these two proteases, t-PA is the most important in plasma (Gale, 2010). The other plasminogen activator is u-PA (Gale, 2010; Medved & Nieuwenhuizen, 2003; Hoylaerts *et al.*, 1982). Both t-PA and u-PA are secreted as single chain proteins. Urokinase-type PA needs to be cleaved by FXIIa, plasmin or kallikrein to its active form, but t-PA is active as a single chain protein (Cesarman-Maus & Hajjar, 2005; Rijken & Sakharov, 2001).

Plasminogen and t-PA bind lysine residues on the surface of a fibrin clot. Plasminogen is then activated by t-PA, forming plasmin, which subsequently cleaves and breaks down fibrin (Gale, 2010). The degradation of fibrin by plasmin leads to the opening of new binding sites (lysine residues) on the surface of the fibrin clot where more t-PA and plasminogen can bind (Rijken & Sakharov, 2001; Higgins & Vehar, 1987). Continuous binding, degradation and more binding subsequently lead to the clot being degraded into small soluble fibrin fragments (Mosnier & Bouma, 2006; Kovlev & Machovich, 2003). Plasminogen is also activated by u-PA in the presence of the u-PA receptor, which is found on various cell types (Gale, 2010).

All three of the pro-enzymes, plasmin, t-PA and u-PA are down-regulated by serine protease inhibitors (serpins) that are present in blood. The serpins therefore act as inhibitors of fibrinolysis. Plasminogen activator inhibitors type -1 and type-2 (PAI-1 and PAI-2) inhibit t-PA and u-PA (Gale, 2010; Rau *et al.*, 2007). When PAI-1 binds to t-PA and u-PA, inert, covalent complexes are formed. This prevents plasminogen from binding to fibrin (Vaughan, 2005). Alpha-2-antiplasmin and α -2-macroglobulin on the other hand inhibit plasmin by binding to the protein (Gale, 2010; Rau *et al.*, 2007). Plasmin is inhibited mostly by α -2-antiplasmin and to a lesser extent by α -2-macroglobulin (Rijken & Sakharov, 2001). Thrombin-activatable fibrinolysis inhibitor (TAFI) is another inhibitor of fibrinolysis. TAFI is a single chain plasma glycoprotein and is activated by thrombin in the presence of thrombo-modulin. TAFI inhibits fibrinolysis by means of preventing binding of plasminogen and t-PA to fibrin through competitive binding of the lysine residues and therefore also the activation of plasminogen to plasmin (Colucci & Semeraro, 2012).

The focus of this study will be on the fibrinolytic aspect of haemostasis. It is possible to measure the ability of the body to lyse clots in different ways. The concentration and/or activity of the individual proteins can be measured, or alternatively global assays can be used which report the time it takes for the body to break down clots in minutes. The PAI-1 activity (PAI-1_{act}) and/or concentration of PAI-1 antigen (PAI-1_{ag}) can for example be measured, acting as proxy markers for fibrinolysis. Alternatively, global fibrinolytic assays (GFA) can be used to measure the global ability of the blood or plasma to lyse clots (Lisman *et al.*, 2001). Several global assays are available; however, due to differences in methodology, not all give a true reflection of plasma clot lysis potential. This will be discussed in more detail in section 4.1. The rest of this literature study will now focus in greater depth on the two fibrinolytic markers, PAI-1_{act} and clot lysis time (CLT) and their relationship to body composition.

2.3 PLASMINOGEN ACTIVATOR INHIBITOR TYPE-1

2.3.1 PAI-1 and its role in CVD

As already mentioned, PAI is a prominent inhibitor of the fibrinolytic pathway through its inhibition of t-PA and u-PA (Diebold *et al.*, 2008; Rau *et al.*, 2007; Lee & Huang, 2005). PAI-1 is part of the serpin family. This serpin family has a common characteristic, namely an arginine in the reactive centre (Diebold *et al.*, 2008; Saksela *et al.*, 1988). Members of this family include PAI-1, PAI-2, PAI-3 (protein C inactivator) and protease nexin 1, all acting as inhibitors of the plasminogen activators t-PA and u-PA, as well as thrombin (Rau *et al.*, 2007; Lee & Huang, 2005). PAI-1 is the most extensively studied serpin member and the most ubiquitous of the two major PAIs. Plasminogen activator inhibitor type-1 regulates both t-PA and u-PA and is considered the main and most rapidly acting physiological inhibitor of plasminogen activation. Two-thirds of plasma t-PA is bound to PAI-1 (Diebold *et al.*, 2008; Rau *et al.*, 2007; Cesarman-Maus & Hajjar, 2005; Lee & Huang, 2005).

The PAI-1 molecule is a single-chain glycoprotein consisting of 379 amino acids and has a mass of 50 KDa (Rau *et al.*, 2007; Cesarman-Maus & Hajjar, 2005; Binder *et al.*, 2002; Saksela *et al.*, 1988). PAI-1 is relatively unstable and has a half-life of 1 -

2 hours in the circulation (Rau *et al.*, 2007; Lawrence *et al.*, 1990). However, it occurs in plasma mainly bound to the extracellular matrix protein, vitronectin. The PAI-1-vitronectin complex has an increased half-life twofold to fourfold (4 – 6 hours) that of PAI-1 alone. It is removed from the circulation mainly by the liver (Rau *et al.*, 2007; Hoekstra *et al.*, 2004; Lawrence *et al.*, 1997). PAI-1 occurs at low concentrations of about 20 ng/ml in the plasma, while platelets account for more than 90% of blood PAI-1 (Booth, 1999). Normal plasma levels for PAI-1 are rather variable, even among healthy individuals, with distinct circadian variations (Kluft *et al.*, 1988). Reported ranges vary between 5 - 80 ng/ml (Binder *et al.*, 2002; Huber *et al.*, 2001). However, despite this variation in ranges, increased elevations in plasma PAI-1 have been observed in various diseases as well as in patients with characteristics that predispose them to CVD, including increased body weight and central fat distribution (Juhan-Vague & Alessi, 1993; Kruithof *et al.*, 1988).

PAI-1 is synthesized in and released from monocytes, macrophages, hepatocytes, adipocytes, platelets, endothelial cells and vascular smooth muscle cells (Rau *et al.*, 2007; Lee & Huang, 2005; Samad *et al.*, 1996). However, the predominant origin of circulating PAI-1 under normal and pathological conditions remains uncertain (Dellas & Loskutoff, 2005).

After vessel injury, activated platelets release PAI-1 and its binding to t-PA serves to stabilize the thrombus and protect it from premature fibrinolysis (Kohler & Grant, 2000). Later in the coagulation process, plasminogen/plasmin and t-PA are bound to fibrin within the thrombus. This protects t-PA from inhibition by PAI-1, resulting in plasmin generation and fibrinolysis (Cesarman-Maus & Hajjar, 2005; Vaughan, 2001).

Due to the inhibitory effect of PAI-1 on fibrinolysis, it has been suggested that PAI-1 may play a role in CVD (Kohler & Grant, 2000). Elevated PAI-1 levels result in more t-PA being bound and this suppresses fibrinolysis. Therefore increased PAI-1 levels have been considered a potential risk factor for CVD and are also associated with the development of myocardial infarction and enhanced progression to thrombosis (Kohler & Grant, 2000; Hawkins, 2004; Hoekstra *et al.*, 2004). Not only has PAI-1 been well established as a contributing pro-thrombotic/anti-fibrinolytic factor in

chronic and acute coronary artery disease, but also in other arterial – and venous thromboembolic disorders (as reviewed by Huber *et al.*, 2001). However, despite the strong body of evidence indicating associations between elevated PAI-1 levels and CVD, its status as a factor is still unclear. Folsom *et al.* (2001) summarised several studies that have found associations between hypofibrinolysis and CVD due to increased PAI-1 levels. However, in many of these studies this association disappeared after adjustment for known CVD risk factors (overweight, a sedentary lifestyle, diabetes mellitus, hypertension, smoking and dyslipidaemia). Therefore, PAI-1 may possibly not be a major (casual) factor in CVD (Hoekstra *et al.*, 2004), but an elevated PAI-1 level seems to be an important CVD risk marker.

2.3.2 Association between PAI-1 and body composition

Various factors have been shown to be determinants of plasma PAI-1 levels, as summarised by Hoekstra *et al.* (2004). These factors include genetic determinants, excessive alcohol consumption, insulin resistance and diabetes, an unfavourable blood lipid profile, decreased oestrogen levels, physical activity, and obesity (Hoekstra *et al.*, 2004). This section will focus on the association between PAI-1 and body composition since it seems that obesity and especially central obesity have been shown to be associated with elevated PAI-1 levels (Nienaber *et al.*, 2008; Hoekstra *et al.*, 2004; Juhan-Vague *et al.*, 2002; Sartori *et al.*, 2001; Ferguson *et al.*, 1998; Juhan-Vague & Alessi, 1993).

PAI-1 is the protein of the haemostatic system that is the most disordered in obesity (Mutch *et al.*, 2001). Obesity is a well-documented independent risk factor for CVD and impaired fibrinolysis has been strongly associated with obesity (Bennet *et al.*, 1966; Fearnley *et al.*, 1963). Elevated PAI-1 levels has been shown to be an important cause of hypofibrinolysis (McGill *et al.*, 1994; Landin *et al.*, 1992; Vague *et al.*, 1989; Vague *et al.*, 1986).

Obesity is associated with an increase in fat mass and thus adipose tissue. Adipose tissue synthesises and directly secretes PAI-1 (Dellas & Loskutoff, 2005; Cigolini *et al.*, 1999; Morange *et al.*, 1999; Samad *et al.*, 1999). Therefore the potential exists that the increased amount of adipocytes in an obese subject would have the capacity

to synthesise PAI-1 that could exceed synthesis by other tissues (Mutch *et al.*, 2001). A higher production of PAI-1 from adipocytes from obese subjects was seen compared to adipocytes from lean subjects, even after adjusting for the size of the adipocytes (Gottschling *et al.*, 2000; Eriksson *et al.*, 1998a). Adipose tissue further increases plasma PAI-1 through increasing the hepatic production of PAI-1 which is stimulated by adipocyte-derived cytokines (tumour necrosis factor-alpha (TNF- α) and tumour growth factor-beta (TGF- β)) (Frühbeck *et al.*, 2001; Morange *et al.*, 1999).

Differences in the release of PAI-1 from different fat depots have been shown from studies on human subjects. Plasma PAI-1 has been shown to be directly associated with visceral fat in obese and non-obese children and adults (Ferguson *et al.*, 1998; Cigolini *et al.*, 1996). PAI-1 secretion by human adipocytes has been reported to be more pronounced in visceral fat than in subcutaneous fat (Bastelica *et al.*, 2002; Cigolini *et al.*, 1999; Alessi *et al.*, 1997). However, more recently it has been shown that it is stromal cells and not the adipocytes themselves that are the most important sources of PAI-1 within adipose tissue (Bastelica *et al.*, 2002). The regional differences in PAI-1 production may be explained by the fact that subcutaneous fat contains much lower amounts of stromal cells compared to higher amounts found in visceral fat (Bastelica *et al.*, 2002).

2.4. GLOBAL PLASMA FIBRINOLYTIC POTENTIAL

It is possible to measure the ability of the body to lyse clots. This can be done in different ways. Assays using whole blood or plasma exist but not all of them include all the clotting and lysis components (Lisman *et al.*, 2001). Therefore, since not all assays reflect the true global potential of the body to dissolve clots (Prins & Hirsh, 1991), it is difficult to draw conclusions from the data. This section of this literature study aims to provide a short description and comparison of the different assays available to determine global fibrinolytic potential.

2.4.1 Different methods for the measurement of global plasma fibrinolytic potential

Assays that use whole blood are able to reflect more truly *in vivo* clot lysis as compared to assays that use plasma only. Whole blood contains cellular components not present in plasma alone (Prins & Hirsh, 1991). Two examples of whole blood clot lysis assays are the dilute whole-blood clot lysis time (DWBCLT) assay and the method for measuring global fibrinolytic capacity (GFC).

The DWBCLT assay is a method used to study the association of thrombosis and hypofibrinolysis (Cellai *et al.*, 2010). However, in this assay, instead of tissue factor, thrombin is used to initiate clot formation and in addition, it is performed in the absence of calcium. Lysis time is therefore independent of the coagulation process and, as a consequence, independent of TAFI. There is an exclusion of the interaction between coagulation and fibrinolysis through TAFI and factor XIII. This is due to the presence of citrate that prohibits the generation of thrombin. Therefore, despite the advantage of a whole blood assay to better reflect *in vivo* clot lysis, this assay is an example of an assay that is not a true reflection of global clot lysis and global fibrinolytic potential (Cellai *et al.*, 2010; Meltzer *et al.*, 2009; Lisman *et al.*, 2005; Lisman *et al.*, 2001).

The method for measuring GFC in whole blood was developed by Rijken *et al.* (2008). In this method blood samples are collected in thrombin with and without aprotinin and clots are formed during incubation. After centrifugation, collected serum is analysed for fibrin degradation products using an enzyme immuno assay. The GFC is assessed from the difference between the fibrin degradation products in the two sera and expressed in $\mu\text{g/ml}$. The disadvantage of using whole blood clot lysis assays is that they have to be performed on fresh whole blood, making the assay impractical in large epidemiological studies, such as the PURE study, in which samples are typically collected and frozen in the field and analysed only at a later stage.

Other assays use plasma to measure fibrinolytic potential. These assays will be described below in order to give examples of the differences in the methods and which components are included and/or excluded. Some assays that use plasma to

measure fibrinolytic potential, do not give a true reflection of global plasma clot lysis time. The euglobulin clot lysis time (ECLT) is an example of a plasma assay where not all the coagulation and fibrinolytic components of the plasma are included (Kowalski *et al.*, 1959). The ECLT method is used to measure plasma fibrinolytic activity in plasma by using an euglobulin fraction of the plasma that is prepared, re-suspended in a solution and maximum absorbance is measured. Lysis time is defined where the lysis curve reaches an absorbance of 0.05 or less (Smith *et al.*, 2003; Kowalski *et al.*, 1959). Lisman and co-workers (2005) stated that the outcome of this test does not reflect a true global fibrinolytic potential. This test is performed with an euglobulin fraction of plasma that is partially depleted from several physiologic fibrinolytic inhibitors and which, as in the case of the DWBCLT assay, also excludes the interplay between coagulation and fibrinolysis through TAFI and FXIII.

Goldenberg *et al.* (2005) developed and investigated a global assay that measures both coagulation and fibrinolysis. In the clot formation and lysis (CloFAL) assay, a reactant solution (containing calcium, tissue factor and t-PA) is added to citrated platelet-poor plasma. Measured changes in absorbance reflect ongoing activation of clot formation and fibrinolysis reactions. The researchers suggest that the CloFAL assay may not be sensitive to PAI-1 as results from PAI-1 deficient plasma did not show the expected brisk decline in absorbance. This highlights possible insensitivity to disorders of haemostasis as a potential disadvantage of this assay (Goldenberg *et al.*, 2005).

The researcher He and co-workers (1999) developed a rapid and simple assay to determine the overall haemostatic potential (OHP) of plasma. In this method citrated plasma is diluted with a mixture containing calcium, thrombin and t-PA and absorbance is measured. This method was later modified so that it can be used for routine laboratory and research work (He *et al.*, 2001). In the modified version, thrombin in a decreased dose, with or without t-PA, is added to plasma for initiation of fibrinogen clotting and absorbance is measured. Where the previous method generated only one curve, two fibrin aggregation curves are now generated: the OHP curve and the overall coagulation potential (OCP) curve. A difference between the two curves reflects the overall fibrinolysis potential (OFP) (He *et al.*, 2001). It

was suggested that both the OCP and OFP be used supplementary to the OHP to provide details regarding changes in the coagulation and/or fibrinolytic system (He *et al.*, 2001).

In the EuroCLOT study, Carter *et al.* (2007) combined a turbidimetric clotting assay and a turbidimetric lysis assay to measure clot formation and lysis. Thrombin instead of tissue factor is used to initiate coagulation. This results in the absence of an interaction between the different factors from the coagulation cascade part of clot formation. The lysis assay is carried out in the same way as the clotting assay but with the addition of t-PA to facilitate fibrinolysis. Citrate plasma is added to a buffer and an activation mix containing thrombin and calcium. For the lysis assay t-PA added before the activation mix is added, whereafter clot formation and lysis are measured. The turbidity curves are used to determine 50% lysis time as well as time to complete lysis (defined as time from maximum absorbance to time absorbance returns to baseline) (Carter *et al.*, 2007).

Other turbidimetric global assays performed with plasma are the methods of Undas *et al.* (2006), Leander *et al.* (2012) and Colucci *et al.* (2008). Undas *et al.* (2006) use thrombin to initiate clotting. This assay was, however, designed to measure lysis and not coagulation, therefore clotting time of clots is not taken into account when lysis time is reported. Leander *et al.* (2012) modified the method of He *et al.* (2001) by replacing the thrombin with tissue factor. Colucci *et al.* (2008) also use tissue factor to initiate coagulation and, compared with other studies, use low t-PA concentrations similar to physiological concentrations to facilitate fibrinolysis. The use of tissue factor in these assays includes many coagulation factors that were previously excluded and the methods seem to be sensitive to fibrinolysis inhibitors PAI-1 and TAFI (Leander *et al.*, 2012; Colucci *et al.*, 2008).

A plasma-based assay that is considered to be a good reflection of global plasma fibrinolytic potential is the assay of Lisman *et al.* (2001), because the outcome of this assay is influenced by levels of proteins both involved in coagulation and fibrinolysis such as plasminogen, α -2-antiplasmin, PAI-1 and TAFI (Meltzer *et al.*, 2009) and also incorporates the interplay between coagulation and fibrinolysis. The addition of tissue factor initiates coagulation, creating an environment where coagulation is

dependent on the coagulation cascade of the body. Exogenously added t-PA is responsible for clot lysis. This mimics release of high amounts of t-PA from endothelial cells in response to injury to the vessel wall (Lisman *et al.*, 2001). Since this method is currently considered to give the most accurate reflection of plasma fibrinolytic potential, this assay was chosen to determine clot lysis time in this study.

2.4.2 Association between fibrinolytic potential and body composition

Various studies in Caucasians have shown an association between plasma CLT and CVD events, with longer CLTs observed in patients with CVD as compared to the controls (Leander *et al.*, 2012; Guimarães *et al.*, 2009; Meltzer *et al.*, 2009; Meltzer *et al.*, 2008; Lisman *et al.*, 2005). Longer CLTs were also found in patients with myocardial infarction by Siegerink and co-workers (2011); however they reported shorter CLTs in stroke patients. In terms of individual CVD risk factors in Caucasians, positive associations between CLT and increased BMI, diabetes, increased TC, triglycerides and CRP have been shown (Guimarães *et al.*, 2009; Meltzer *et al.*, 2009, Meltzer *et al.*, 2010a). Until recently no information was available regarding the relationship between CLT and CVD risk factors in Africans. In a recent investigation by De Lange *et al.* (2012), it was found that the main predictors of CLT in black South Africans from the PURE population were PAI-1_{act}, BMI, alcohol consumption and HbA1c.

Since a relationship between CLT and BMI as an anthropometric measure of body composition as a CVD risk factor has been described in the literature, and since the purpose of this study is to investigate the relationship between markers of fibrinolysis and body composition in black adults, a more detailed discussion of the association between fibrinolytic potential and body composition will now follow.

In a population-based case-control study, Meltzer *et al.* (2008) determined fibrinolytic potential in patients with venous thrombosis. In the control group results showed a positive relation between BMI and CLT. CLTs increased substantially with increasing BMI, with an increase of 2.0 min in CLT per 1 kg/m² increase in BMI (Meltzer *et al.*, 2008). A positive relation between CLT and diabetes was also found. The researchers suggested that PAI-1 may play a role in these associations.

In another study by Meltzer *et al.* (2009), CLT as a risk factor for myocardial infarction and arterial thrombosis was measured in 421 male patients and 642 controls. A strong association between CLT and BMI confirmed the same findings from their previous study (Meltzer *et al.*, 2008). Again increased PAI-1 levels were considered as an explanation for this association although PAI-1 was not measured in this study. The researchers (Meltzer *et al.*, 2009) mentioned that PAI-1 is considered to be a biomarker of visceral fat storage (as reviewed by Alessi *et al.*, 2007). They also offered TAFI as an explanation for the association, since TAFI levels have been associated with lipid levels and BMI (Aubert *et al.*, 2003; Silveira *et al.*, 2000).

Guimarães *et al.* (2009) investigated the relationship between defective fibrinolysis and arterial thrombosis. The plasma fibrinolytic potential of 335 young survivors of a first arterial thrombosis was determined. Compared to the controls, the patients exhibited significantly longer CLTs. Also, obese individuals presented with elevated CLTs. A linear trend was observed between CLTs and BMI as well as between age and BMI in this study population. In addition, longer CLT was also associated with diabetes. The researchers suggested the involvement of PAI-1 in the observed effects. This may be possible since PAI-1 levels correlated with advancing age, body mass, plasma insulin levels and the presence of diabetes and it is known that PAI-1 is over-expressed in patients with diabetes and obesity (Guimarães *et al.*, 2009; Alessi & Juhan-Vague, 2006).

The recent investigation by De Lange *et al.* (2012) showed that PAI-1_{act} (27%) and BMI (8%) were two of the main contributors of the variance in CLT in black South Africans from the PURE study population. The researchers also reported that CLT correlated better with BMI (an indicator of overall obesity) than with waist circumference (WC) (an indicator of visceral fat/central obesity). This association between BMI and CLT remained after adjustment for PAI-1_{act}. This is noteworthy since in the same population, PAI-1_{act} (a major predictor of CLT) had a significantly stronger correlation with WC than with BMI (Pieters *et al.*, 2010). As mentioned previously in this literature study, the relationship between PAI-1 and WC is likely due to the fact that visceral adipose tissue is a major source of PAI-1 production and secretion (Bastelica *et al.*, 2002). Plasma PAI-1 levels are further increased by

hepatic production in response to adipocyte-derived cytokines (Frühbeck *et al.*, 2001; Morange *et al.*, 1999). According to De Lange *et al.* (2012), while CLT seems to be related to general obesity, PAI-1_{act} seems to be more related to abdominal obesity. The researchers further indicated that although increased PAI-1_{act} levels are likely to play an important role in the relationship between CLT and general body fat, there might also be other mechanisms that may explain this association, not related to the link between PAI-1 and visceral fat.

2.5 PHYSICAL ACTIVITY AND FIBRINOLYSIS

Also of interest is the potential link between PAI-1 and CLT as markers of fibrinolysis and physical activity. Studies investigating the relationship between physical activity and CLT are scarce, but more literature regarding the relationship between physical activity and PAI-1 levels is available. From a systematic review by Lee and Lip (2003) it was concluded that there are important differences between the effects of moderate endurance physical training and short-term strenuous exercise on both haemostatic and fibrinolytic variables

Two studies where substantial improvements in PAI-1 levels with increasing physical activity were demonstrated have, however, also involved a dietary modification programme (Lindahl *et al.*, 1998; Lindahl *et al.*, 1999). It is thus impossible to arrive at a conclusion about the independent effects of increased physical activity on PAI-1 levels. Interestingly, in both studies significant weight loss was accompanied by reduced PAI-1 levels and although not significant, reduced plasma insulin levels (Buchan *et al.*, 2012). However, studies investigating the independent effects of increased physical activity on PAI-1 levels are limited. Since information about physical activity is available for the follow-up South African PURE data, the relationship between markers of fibrinolysis and physical activity will also be investigated in this study.

2.6 CONCLUSION

The prevalence of CVD is increasing in black South Africans. A significant health transition due to urbanisation is contributing to this increased CVD prevalence since

urbanisation is associated with significant changes in diet and physical activity. These changes negatively impact on CVD risk factors, contributing to the higher incidence of these risk factors in the black South African population such as obesity and hypofibrinolysis in women. Limited information is available regarding the haemostatic profile of black South Africans in relation to CVD risk. From the PURE 2005 data, it is clear that PAI-1 and CLT as markers of fibrinolysis, associated in different ways with markers of body composition, despite the strong relationship that existed between PAI-1 and CLT. The remainder of this mini-dissertation will focus on the further exploration of this observation. The association of PAI-1_{act} and CLT as markers of fibrinolysis and different markers of body composition and physical activity in the PURE 2010 population will be investigated.

CHAPTER 3: **METHODOLOGY**

3.1 INTRODUCTION

From the PURE 2005 data it was clear that PAI-1 and CLT associated differently with markers of body composition despite the strong relationship that exists between PAI-1 and CLT. It was decided to explore this observation further in the PURE 2010 data set for which much more detailed body composition data are available. Therefore, the main aim of this study is to investigate the association of PAI-1_{act} and CLT as markers of fibrinolysis with a variety of body composition markers, as well as to investigate the relationship between PAI-1_{act}, CLT and physical activity, in the South African PURE data collected during 2010. Specific objectives were:

1. To measure PAI-1_{act} in the South African PURE population (2010) (n = 1288).
2. To measure CLT as a marker of global plasma fibrinolytic potential (Lisman *et al.*, 2005), in the South African PURE population (2010) (n = 1288).
3. To investigate the relationship between:
 - 3.1 PAI-1_{act} and markers of body composition including BMI, WC, waist: height ratio, waist: hip ratio (WHR) and skinfolds (triceps, chest, abdominal, thigh and supra iliac skinfolds) in the total study population (n = 1288);
 - 3.2 PAI-1_{act} and body fat percentage, fat mass and fat-free mass in an urban subgroup of the study population (n = 144) by means of air-displacement plethysmography (ADP) using a *BodPod[®]; and in the rural group (n = 697) by means of bioelectrical impedance analysis (BIA) using a *Tanita Ironman[™].
4. To investigate the relationship between:
 - 4.1 plasma fibrinolytic potential (expressed as clot lysis time (CLT)) and markers of body composition including BMI, WC, waist: height ratio, WHR and skinfolds (triceps, chest, abdominal, thigh and supra iliac skinfolds) in the total study population (n = 1288);

- 4.2 global plasma fibrinolytic potential and body fat percentage, fat mass and fat-free mass in an urban subgroup of the study population (n = 144) by means of ADP using a *BodPod[®]; and in the rural group of the study population (n = 697) by means of BIA using a *Tanita Ironman[™].
5. To compare the relationship between PAI-1_{act} and the abovementioned markers of body composition with the relationship between global plasma fibrinolytic potential and the abovementioned markers of body composition.
6. To investigate the relationship between PAI-1_{act}, CLT and physical activity in the study population (n = 1288).

*The BodPod[®] could only be used on an urban subgroup of 144 participants owing to practical reasons. After 144 urban participants were measured the instrument broke down and it was replaced with the Tanita Ironman[™] that was sent from Canada. Unfortunately by the time it reached South Africa it was too late to measure the rest of the urban participants. Another reason for the BodPod[®] being used only in the urban group was that this is a very sensitive instrument and it could not be transported over long distances to the rural areas. Rural-urban comparisons will not be made for these anthropometrical variables in view of the two different measurement techniques used.

This chapter provides a detailed description of the ethical approval, study design, recruitment and characteristics of the participants. This is followed by a description of the study procedure and data collection tools. The experimental methods used to analyse the samples as well as the statistical analysis of the data are then explained in full. The analysis of the blood samples was done in 2011 and 2012.

3.2 ETHICAL CONSIDERATIONS

The Ethics Committee of the North-West University approved this study and gave it the ethics number NWU-00016-10-A1 with the date of ethical approval as 23 February 2010 (Addendum A). The purpose of the study and all study procedures were explained to the participants in their home language before informed consent

was obtained. Participants signed an informed consent declaration (Addendum B) before being recruited and the study commenced. Participants had the option to withdraw from the study at any time.

3.3 STUDY DESIGN

The overall PURE study was coordinated from Canada as a large-scale international epidemiological cohort study. The study tracks changing lifestyles, risk factors and chronic disease over 12 years using periodic standardised data collection in urban and rural areas of seventeen low, middle and high-income countries in transition (including South Africa) from around the world. Data are collected on national, community, household and individual level (Teo *et al.*, 2009). The present study investigated the association between fibrinolysis markers and body composition within the South African PURE cohort, using data of participants that participated in the follow-up study during 2010.

3.4 STUDY POPULATION AND PROCEDURES

3.4.1 Recruitment of participants

For the South African arm of the International PURE study, a census of 6000 African households was undertaken in 2005, starting from a randomly selected address in each of four (two rural and two urban) communities in the North-West Province. The two rural communities, both still under tribal law, included Ganyesa (informal town in rural setting) and Tklagameng (rural). The urban communities, residing in informal and formal settlements surrounding the city of Potchefstroom, were Ikageng (established urban) and Zonderwater, extensions 7 and 11 (informal settlements). All the baseline data for South Africa were collected during 2005.

The household census was conducted in 6000 houses (1500 in each community) by trained field workers. The household census at family level included two questionnaires. The family questionnaire included questions regarding number of people in the household, their ages and health profile, in order to identify all

members eligible to participate in the study. The household questionnaire contained questions regarding the socio-economic status of the household. Every head of household gave signed consent to fill out the questionnaires. For households where the head of the household was not at home or refused to take part in the study, non-responder questionnaires were completed. The next house was then taken and the procedure was repeated until 1500 households were included in a specific community. From the obtained data of the 6000 households, a paper selection of possible participants according to specific eligibility criteria (see section 3.4.2) was made. One thousand participants from each community, thus 2000 participants from the rural settlements and 2000 participants from the urban settlements were selected, with a total of 4000 participants. After a home visit to the 4000 participants, and after giving informed consent, an extensive standardised interviewer-administered adult questionnaire regarding physical and psychological health, socio-economic background, lifestyle practices and support systems available was compiled. A total of 3750 questionnaires were completed. From the 3750 questionnaires completed at individual level, a further selection of 2792 questionnaires was made (1444 rural and 1348 urban participants respectively) based on voluntary inclusion, availability during the blood collection period and after having indicated that they had no plans to relocate in the foreseeable future.

3.4.2 Eligibility criteria

Inclusion criteria for participants to be eligible for the study were apparently healthy male and female volunteers 35 years and older, residing in the households that were approached. Household members (usual and migrant residents) of rural and urban households were included in the study. A usual resident was defined as a person who eats and sleeps in a household most days of the week and most weeks of the year, this person considers the household his/her primary place of habitation over the long term. Renters, boarders and servants were not considered household members. Migrant residents were defined as individuals who live in a household less than two days per week, but consider the household as their primary place of habitation. These individuals eat or sleep elsewhere due to the need to work, study or train in a different physical location. Exclusion criteria related to individuals who did not give consent to participate in the study. In terms of health, the volunteer

should not have used medication for chronic disease (except hypertension) and/or not have any known chronic condition or disease (except hypertension) including tuberculosis and HIV infection. Since the South African PURE study was planned to run for 12 years, participants who were planning to move away from the study area in the foreseeable future, were also excluded from the study.

3.4.3 Study procedures

From the 2792 questionnaires selected, 2010 apparently healthy participants were eventually prospectively enrolled and participated in the study. Reasons for this reduced number from 2792 were that some participants were not available during the blood collection period despite having indicated their availability; others had an acute illness such as a cold and were therefore excluded from the study. Data for these individuals were not collected at a later stage due to financial constraints and the remoteness of the study site. The 2010 black South African participants included 1260 women and 750 men from the rural (n = 1006) and urban (n = 1004) settlements. During the period August until November 2005, each of the 2010 participants again gave informed consent for the measurement of several physical measures (blood pressure, anthropometric measures, lung function and ECG), as well as for a urine and blood sample to be taken. All participants were tested for human immunodeficiency virus (HIV) infection, but were given the option of whether they wanted to know their status or not. Prior to the blood sample being taken, all participants received pre-test counselling in groups of 10. Post-test counselling was done individually when participants received results. They also received feedback on results from blood pressure, lung function, ECG and blood glucose measurements before going home. All participants identified with an abnormality regarding tested markers, received a referral letter to the nearest clinic or hospital. Data on physical activity levels and habitual dietary intakes were also assessed by questionnaires.

In 2010, the first follow-up in the PURE study was conducted. Of the 2010 participants who were included in the South African PURE baseline study in 2005, only 1288 participants (rural n = 699; urban n = 589) returned for the study visits and were then included in the PURE 2010 follow-up study. Of the 1288 participants

there were 435 men and 853 women. Reasons for the decrease in the number of participants from 2005 to 2010 included: 1) death of participants (n = 233), 2) participants that moved away outside the study area, 3) participants that refused to participate or refused to have a blood sample taken, 4) participants that failed to show up on the day of blood collection, 5) participants withdrawing from the study and, 6) participants lost to follow-up.

After recruitment, all participants received HIV pre-counselling whereafter voluntary and informed consent (Addendum B) was obtained. A blood sample was taken from each participant. All participants were again tested for HIV infection, and were given the option whether they wanted to know their status or not. Post-test counselling was done individually. After the blood sample was taken, several physical measurements were taken for each participant. These physical measures included: blood pressure, radiological investigation (sonar) and measures of body composition which included anthropometric measures, air-displacement plethysmography (ADP) and bioelectrical impedance analysis (BIA) which will be discussed in more detail in the following sections.

After the physical measures were taken, several questionnaires were administered: an extensive standardised interviewer-administered adult questionnaire, a physical activity questionnaire, a diet food frequency questionnaire and a psychological questionnaire. Participants received feedback regarding results for HIV testing, blood pressure and blood glucose. Participants received referral letters to the nearest clinic or hospital when identified with an abnormality regarding tested markers.

For this mini-dissertation, data of the follow-up South African PURE study population collected in the year 2010 were used, therefore only methodologies regarding collection of data used in this study will now be discussed in more detail. The adult and physical activity questionnaires will be discussed in section 3.5 and blood pressure measurement in section 3.6. All body composition measures relevant to this study will be discussed in section 3.7 and blood sampling and relevant biochemical analyses will be addressed in section 3.8 and section 3.9 respectively. This chapter will end with an explanation of the statistical analysis of the data.

3.5 QUESTIONNAIRES

3.5.1 Adult questionnaire

During data collection in 2010, an extensive standardised interviewer-administered adult questionnaire (Addendum C) was completed for each participant. Trained fieldworkers received instructions on how to administer and complete the questionnaires. The first part of the questionnaire addressed demographical information from the participants such as: gender, age, ethnicity, marital status, education level, occupation and employment status. Questions regarding current habits (smoking tobacco, use of alcohol and medication) followed, as well as questions about current physical and health status, especially occurrence of events or new diagnoses since the last visit in 2005. The next group of questions focused on the psychological health, socio-economic background and lifestyle practices and support of the participant. Lastly the questionnaire contained questions regarding the participant's attitude towards HIV or acquired immune deficiency syndrome (AIDS).

3.5.2 Physical activity questionnaire

The physical activity questionnaire (Addendum D) that was administered is based on a short questionnaire developed by Baecke *et al.* (1982), but was adapted for use in black South Africans (a population in transition) and validated for the THUSA study (Kruger *et al.*, 2000). The questionnaire was designed to measure habitual physical activity that included time spent in the main occupation (employment or housework for the unemployed), stair climbing, commuting, sleep, sport and leisure-time. All types of activity reported by the participants in the questionnaire were coded and used to calculate a physical activity index (PAI) score for each participant. The maximum PAI score that could be obtained was 10.0 that represented an active occupation involving 'sweat-work', climbing stairs daily, participating in vigorous sports activities year-round, standing leisure-time activity such as gardening as well as commuting on foot or bicycle at a vigorous pace. Based on the PAI, participants were then grouped into one of three categories: most active for a PAI score > 6.67; moderately active for a PAI score between 3.34 to 6.67 representing an occupation

which for more than half of the working day involved standing activities, some stair climbing, participating in light sports activities for less than 6 months per year, some standing leisure-time activity as well as commuting on foot or bicycle at a leisurely pace; and inactive for a PAI score between 1.0 and 3.33 representing a sedentary occupation with standing activities for less than half of the day, commuting by car or no commuting, seldom climbing stairs, no sports participation and mostly sedentary leisure-time activity, such as watching television. The minimum PAI score that could be obtained was 1.0 (Kruger *et al.*, 2003; Kruger *et al.*, 2002; Baecke *et al.*, 1982).

3.6 BLOOD PRESSURE MEASUREMENT

Members of the Hypertension in Africa Research Team (HART) of the North-West University, Potchefstroom, were responsible for blood pressure measurements. Blood pressure was measured using the Omron automatic digital blood pressure monitor (Omron HEM-757). Before blood pressure was taken, the participant had to have been resting and calm for more than 5 minutes, should not have smoked, exercised or eaten in the last 30 minutes, and should not have climbed the stairs in the last 15-30 minutes before the measurement was taken. The measurement was taken with the participant seated upright and relaxed with his/her right arm supported at heart level and using the brachial artery. The readings for both systolic and diastolic pressures were recorded.

3.7 BODY COMPOSITION MEASURES

3.7.1 Anthropometric measurements

All anthropometrical measurements were done using the guidelines adopted at the National Institute of Health (NIH) sponsored Arlie Conference (Lohman *et al.*, 1988), with participants wearing minimal clothing. Data of the weight, height, waist circumference (WC), hip circumference and triceps, chest, abdominal, thigh and supra iliac skinfold measurements were used in the present study. The methodologies of these anthropometric measurements will now be explained in more detail.

3.7.1.1 Weight

Weight in kilograms was measured using a portable electronic scale (Precision Health Scale, A&D Company, Tokyo, Japan). Before each measurement it was ensured that the scale was stable on a horizontal floor and that the reading was “0” (zero). The scales were calibrated on a daily basis using standard weights. Participants were weighed wearing minimal clothing, barefoot with arms hanging freely at the sides (Lohman *et al.*, 1988).

3.7.1.2 Height

A calibrated stadiometer was used to measure height with the participants standing upright, arms hanging freely at the sides, shoulders relaxed, with legs straight and knees together. The participants had to be barefoot and standing with their feet flat and heels together. The shoulder blades, buttocks and heels had to be in contact with the stadiometer. The head of the participant was facing straight forward and held in the horizontal Frankfort plane. The horizontal Frankfort plane is represented by a line between the lowest point on the margin of the orbit (the bony socket of the eye) and the tragion (the notch above the tragus, the cartilaginous projection just anterior to the external opening of the ear). Height was recorded to the nearest 0.1 cm after the participant inhaled fully and maintained the erect position without altering the load on the heels (Lohman *et al.*, 1988).

3.7.1.3 Waist circumference

Waist circumference was measured using a non-stretchable standard tape measure (Lufkin, Cooper Tools, Apex, NC, USA) attached to a spring balance exerting a force of 750 gram. The participant had to stand erect with the abdomen relaxed and arms at the sides. The measurement was taken over an unclothed abdomen at the smallest diameter between the costal margin and the iliac crest (the hip), with the tape kept in a horizontal line. The measurement was recorded to the nearest 0.1 cm at the end of a normal expiration (Lohman *et al.*, 1988).

3.7.1.4 Hip circumference

A non-stretchable standard tape measure (Lufkin, Cooper Tools, Apex, NC, USA) attached to a spring balance exerting a force of 750 gram was used to measure hip circumference. The participant had to stand erect with the abdomen relaxed and arms at the sides. The measurement was taken over minimal clothing at the level of the greater trochanters (usually the widest diameter around the buttocks) and the tape had to be kept horizontal. The measurer had to squat at the side of the participant so that the level of maximum extension of the buttocks could be seen. The measurement was recorded to the nearest 0.1 cm (Lohman *et al.*, 1988).

3.7.1.5 Skinfolds

The thickness of all skinfolds were measured with a Harpenden calliper (Baty International West Sussex, UK) with the participant standing, arms hanging loosely and comfortably at the sides, except for the thigh skinfold. The thigh skinfold was measured with the participant seated with an erect upper body, the arms supporting the hamstring, and the leg on which the skinfold was performed extended. After identification of the landmark specific to a skinfold, it was marked with a pen on the skin. The thumb and index finger of the left hand were used to pick up the double layer of skin (skinfold) at the site of the landmark whereafter the skinfold was then gently pulled away from the body (ensuring that underlying muscle was not included in the double layer of skin). With the calliper in the right hand, the head of the calliper was placed perpendicular to the skinfold with the dial facing up, and about 1 cm below the landmark where the double layer of skin was picked up. The calliper pressure was then gently and fully released. The pinch was maintained while reading the dial within 3 - 4 seconds after releasing the calliper pressure. All skinfold measurements were repeated twice and recorded to the nearest 0.1 mm (Lohman *et al.*, 1988).

- **Triceps skinfold**

The triceps skinfold site was located midway between the lateral projection of the acromion process of the scapula and the olecranon process of the ulna with the elbow of the right arm flexed at 90 degrees. The skinfold was measured vertically at

the midline of the posterior aspect of the right arm, over the triceps muscle, at the mid-point of the arm (Lohman *et al.*, 1988).

- Chest skinfold

The chest or pectoral skinfold is an oblique skinfold taken as high as possible on the anterior axillary fold at the pectoral skinfold site. This site is located along the borderline of the pectoralis major muscle between the anterior axillary fold and the nipple (Lohman *et al.*, 1988).

- Abdominal skinfold

The abdominal skinfold measurement was taken vertically at the abdominal skinfold site. This site was located 5 cm horizontally to the right hand side of the omphalion (midpoint of the navel). Care was taken not to place the fingers or the calliper inside the navel (Lohman *et al.*, 1988).

- Thigh skinfold

This vertical skinfold was taken parallel to the long axis of the thigh at the thigh skinfold site. The thigh skinfold site was located at the mid-point of the linear distance between the inguinal point and the patellare. The inguinal point is the point at the intersection of the inguinal fold (the crease at the angle of the trunk and the anterior thigh) and the mid-line of the anterior thigh. The patellare is the midpoint of the posterior superior border of the patella (Lohman *et al.*, 1988).

- Supra iliac skinfold

The supra iliac skinfold measurement was taken with the double skinfold running obliquely, medially downward and anteriorly at about a 45° angle as determined by the natural fold of the skin, at the supra spinale skinfold site. The supra spinale skinfold site is the point at the intersection of the following two lines: 1) the line from the marked iliospinale landmark to the anterior axillary border, and 2) the horizontal line at the level of the marked iliocristale landmark. The iliospinale landmark is the most inferior or undermost part of the tip of the anterior superior iliac spine. The iliocristale landmark is the point on the iliac crest where a line drawn from the mid-

axilla (middle of the armpit), on the longitudinal axis of the body, meets the ilium (Lohman *et al.*, 1988).

3.7.1.6 Body mass index

Body mass index (BMI) was calculated by dividing weight (kg) by the height (m) that is squared (kg/m^2).

3.7.2 Air-displacement plethysmography

In an urban subgroup ($n = 144$) body composition measurements (body fat percentage, fat mass and fat-free mass) were determined by means of ADP (McCrorry *et al.*, 1995) using the BodPod[®] body compositions system (Model 2000A, Life Measurement Instruments, COSMED, Rome, Italy). ADP has been shown to be an accurate method for assessing body composition (Biaggi *et al.*, 1999). The BodPod[®] body compositions system is a single egg-shaped device consisting of two chambers: a reference chamber, with the breathing circuit and a test chamber, where the participant is seated. The system determines body volume through an air displacement method through the application of Poisson's gas law (McCrorry *et al.*, 1995).

The participants were weighed to the nearest 0.01 kg with the electronic scale of the BodPod[®] system while wearing minimal clothing. Participants were required to wear tight-fitting underwear or a swimsuit as well as a latex swim cap in order to avoid a measurement error contributed to by the hair of the participants. After a two-point calibration with the chamber empty and with a standard 50 litre cylinder in the test chamber, the body volume of each participant was obtained while seated quietly in the test chamber and breathing normally. Two measurements of body volume which make up a single ADP procedure were performed on each participant. If these two measurements differed by more than 150 ml, a third measurement was performed. The body composition parameters (body fat percentage, fat mass and fat-free mass) were calculated according to age and gender and from the results obtained from the BodPod[®].

3.7.3 Bioelectrical impedance analysis

In the rural group (n = 697) body composition measurements of the lower extremities (body fat percentage, fat mass and fat-free mass) were determined by means of BIA. The BIA technique is based on the principle that electrical current flows at different rates through the body depending on its composition. The body is composed mostly of water through which an electrical current can flow. However the body is also composed of body fat (a non-conducting material) and protein, that include muscle and bone (more conductive than body fat). Body fat is a significantly less conductive body compartment than body protein, and therefore provides resistance to, or impedes the flow of electrical current. Impedance (opposition to flow of the current) is a drop in voltage when a low-level current with a fixed frequency passes between electrodes spanning the body. Predictive equations are then used to estimate total body water, fat-free mass, fat mass and body cell mass using gender, age, weight, height and race (Dehghan & Merchant, 2008; Kyle *et al.*, 2004).

The Tanita Ironman™ (Inner Scan body composition monitor) (Model BC-554, Tanita Corporation, Tokyo, Japan) was used to measure body composition for this study. The Tanita body composition analyser is a simplified version of BIA technique and it uses leg-to-leg BIA. It is a precision electronic scale platform with footpad electrodes and therefore the measurements are taken while standing barefoot on the footpad electrodes. The participants were weighed in light clothing and all jewellery in contact with the skin was removed. The body fat monitor automatically measures weight and then impedance. The body fat percentage is then calculated by the computer software and equation formula, making use of the measured impedance, participant gender, height, fitness level, and in some cases age. These participant details are keyed in before the participant stands on the footpads. The Tanita equations are generalised for standard adults, athletes and children.

3.8 BLOOD SAMPLING

A 90ml overnight fasting (at least 8 hours without any food or drink, excluding water) blood sample was collected from each participant by qualified nursing staff. The blood sample was collected from the ante-cubital vein in the right arm of the participant with the use of a disposable needle before 11:00 in the morning. Each

collection tube was filled to maximum capacity to ensure an optimal blood: anticoagulant ratio and gently inverted 5 times to ensure thorough mixing of contents. For the determination of the lipid profile and high-sensitivity CRP, blood was collected in tubes without anticoagulants and allowed to clot at room temperature. For the determination of plasma glucose, blood was collected in fluoride tubes. For the determination of fibrinolytic potential, and PAI-1_{act}, plasma samples were prepared by collecting blood in 3.8% citrate tubes. All blood samples were centrifuged at 2000 × g for 15 minutes at 10°C within 30 minutes after collection. Serum and plasma were aliquotted, frozen on dry ice, stored in the field at -18°C and then after 2 – 4 days at -82°C in bio-freezers until further analysis.

3.9 BIOCHEMICAL ANALYSES

3.9.1 Determination of lipid profile

The analysis of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride were done at the North-West University, Potchefstroom, using the Cobas Integra 400 Plus (Roche, Basel, Switzerland). Both TC and triglyceride were determined by enzymatic colorimetric methods and HDL-C by a homogeneous enzymatic colorimetric assay. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula (Friedewald *et al.*, 1972).

$$\text{LDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol} \\ - (\text{triglycerides}/5)$$

3.9.2 Determination of high-sensitivity C-reactive protein

The analysis of high-sensitivity CRP was done at the North-West University, Potchefstroom. A high sensitivity particle enhanced turbidimetric assay using the Cobas Integra 400Plus (Roche, Basel, Switzerland) was used to measure high-sensitivity CRP.

3.9.3 Determination of plasma glucose

The hexokinase method using the Synchron[®] System (Beckman Coulter Co., Fullerton, CA, USA) and reagents was used to determine plasma glucose concentration. By means of this system, samples and reagents are automatically divided into cuvettes with a ratio of one part sample to 100 parts reagent. Change in absorbance was relative to the glucose concentration of the reagent in the sample, monitored at 340 nm. Plasma glucose concentration was calculated and expressed by means of the change in absorbance.

3.9.4 Determination of PAI-1 activity

PAI-1 is measured as either PAI-1 activity (PAI-1_{act}) or as PAI-1 antigen (PAI-1_{ag}) in human plasma. For this study, the two-stage, indirect chromogenic assay, Spectrolyse[®] PAI-1 Activity Assay (Sekisui Diagnostics, LLC, Stamford, Connecticut, USA), was used to measure PAI-1_{act} in human plasma. This assay is based on the methods as described by Eriksson *et al.* (1988) and Chmielewska *et al.* (1983). One unit of PAI-1_{act} is defined as the amount of PAI-1 that inhibits one International Unit (IU) of human t-PA.

In the first stage of the assay, a fixed amount of human t-PA is added to the plasma sample and allowed to react with the PAI-1 present in the sample. Next the sample is acidified to destroy α -2-antiplasmin that would otherwise interfere with the assay as it is a potential plasmin inhibitor. Subsequently the sample was diluted.

In the second stage, the residual t-PA activity is measured by adding the sample to a mixture of human glu-plasminogen, poly-D-lysine and a chromogenic substrate for plasmin (plasminogen activator reagent (PAR)). The residual t-PA activity in the sample catalyses the conversion of plasminogen to plasmin, which in turn hydrolyses the chromogenic substrate. Poly-D-lysine is present as a stimulator of this t-PA catalysed conversion of plasminogen to plasmin. As the PAI-1_{act} measured in the sample is based upon the residual t-PA activity, the absorbance of the assay is inversely proportional to the amount of PAI-1_{act} in the sample. A detailed explanation of this experimental method can be found in Addendum E.

3.9.5 Determination of plasma fibrinolytic potential

The plasma fibrinolytic potential of tissue factor induced clots was determined. Lysis was accomplished by the addition of exogenous t-PA with the method of Lisman *et al.* (2005) but with slightly modified tissue factor and t-PA concentrations. The method has been validated by Talens *et al.* (2012). Lysis was studied by monitoring the changes in turbidity during clot formation and changes in turbidity during clot lysis using a Multiskan Ascent spectrophotometer (Labsystems, Virginia, USA). Final clot concentrations were tissue factor (59 pM as determined by Duckers *et al.* (2010); Dade Innovin, Siemens Healthcare Diagnostics Inc., Marburg, Germany), CaCl₂ (17 mM), t-PA (100 ng/ml; Actilyse, BoehringerIngelheim, Ingelheim, Germany) and phospholipids vesicles (10 µM; Phospholipids-TGT, Rossix, Mölndal, Sweden). A final t-PA concentration of 100 ng/ml was selected to induce CLTs of between 60 - 100 min using normal plasma. Tissue factor concentration was slightly modified to create a lag phase and to achieve clotting times of between 7 – 8 minutes (De Lange *et al.*, 2012). CLT was defined as the time from the midpoint of clear to maximum turbidity transition, representative of clot formation (clotting time), to the midpoint of maximum turbidity to clear transition, representative of the lysis of the clot (lysis time) (Lisman *et al.*, 2005). Clotting and lysis times were measured by sigmoidal curve fitting using the computer program Origin version 8.5 (OriginLab Corporation, Northampton, MA, USA). A detailed explanation of this experimental method can be found in Addendum F.

3.10 STATISTICAL ANALYSIS

Data were analysed with the computer software package Statistica[®] (Statsoft Inc., Tulsa, Oklahoma, USA). A *P*-value of less or equal to 0.05 was regarded as statistically significant. Normally distributed data were expressed as mean (95% confidence intervals). Not normally distributed data were log-transformed to improve normality to enable use of parametric statistics. Pearson as well as partial correlations were used to determine the relationships / associations between fibrinolysis markers (PAI-1_{act} and global plasma fibrinolytic potential) and markers of body composition. Significant differences between correlation coefficients obtained for the correlation of PAI-1_{act} with body composition markers and the correlation coefficients obtained for CLT and body composition markers were also calculated.

T-tests and analysis of variance with Tukey's honest significant difference post-hoc tests, were used to compare PAI-1_{act} and global plasma fibrinolytic potential between different sub-groups. Analysis of co-variance (ANCOVA) was used to take possible cofounders into consideration. Least square means (95% CI) for normally distributed and least square geometric means (95% CI) for non-parametric data are reported when comparing sub-groups.

CHAPTER 4: RESULTS

4.1 INTRODUCTION

This chapter presents the baseline characteristics of the PURE 2010 study population and correlations of markers of fibrinolysis (PAI-1_{act} level and CLT) with different body composition and physical activity variables for men and women separately. Differences in markers of fibrinolysis (PAI-1_{act} level and CLT) between tertiles and categories of different body composition variables within and between genders are also presented.

4.2 PARTICIPANT CHARACTERISTICS

Table 4.1 presents the baseline characteristics of the study participants. In this arm of the South African PURE study, a total of 1288 Africans participated. Of the 1288 participants, 66.2% were women (n = 853) and 33.8% were men (n = 435). The mean age of the participants was 55.0 years (median 53.7 years) with no significant difference between genders (p = 0.42).

The BMI was not normally distributed for the total group with a median of 24.0 (interquartile range (IQR) 19.8 - 30.2) kg/m². There was a significant difference (p < 0.0001) between the BMI of the men and women. The median BMI of the total group as well as the BMI of the men (20.2 kg/m²) fell within the normal weight ranges for healthy adults of 18.5 - 24.9 kg/m² (WHO, 2006). The median BMI of the women of 27.0 kg/m² fell within the reference range of 25.0 to 29.9 kg/m² that is indicative of overweight or pre-obesity (WHO, 2006). Of the women, 24.5% were overweight and 35.6% were obese, while only 12.0% of the men were overweight and 4.9% were obese (Tables 4.4 and 4.5). The median WC of the men and women was 75.5 (IQR 70.0 – 83.6) cm and 83.0 (IQR 73.1 – 93.5) cm respectively with a significant difference between the genders (p < 0.0001). Internationally recommended WC thresholds for abnormal waist circumferences that indicate abdominal obesity in sub-Saharan Africans are ≥ 94.0 cm for men and ≥ 80.0 cm for women (Alberti *et al.*, 2009; Alberti *et al.*, 2006; Grundy *et al.*, 2005). Compared to these international cut-

off points, the median WC of the men fell below the abdominal obesity threshold, whereas the median WC of the women was above the cut-off. However, two studies suggested the use of other WC cut-offs to indicate central obesity in Africans (Crowther & Norris, 2012; Motala *et al.*, 2011). Using these cut-offs, the median WC of both the men and the women was below the recommended cut-off points of 86.0 cm for men and 92.0 cm for women respectively (Crowther & Norris, 2012; Motala *et al.*, 2011).

A waist: height ratio of ≥ 0.5 indicates central fat distribution (Hsieh *et al.*, 2003). The median waist: height ratio for the total group was 0.51 (IQR 0.44 – 0.57) and for the women 0.53 (IQR 0.47 – 0.59). The men had a significantly lower waist: height ratio of 0.45 (IQR 0.42 – 0.51) ($p < 0.0001$). The median WHR of the men was 0.94 (IQR 0.88 – 0.98) and it differed significantly ($p < 0.0001$) from that of the women of 0.86 (IQR 0.81- 0.90).

Compared to the men, the women had significantly ($p < 0.0001$) higher values for % body fat as measured with both air-displacement plethysmography (ADP) using a BodPod[®] and bioelectrical impedance analysis (BIA).

The median physical activity score of the total group was 2.96 (IQR 2.63 – 3.17) which fell within the range of 1.0 and 3.33 that represents inactivity or a sedentary occupation (Kruger *et al.*, 2003; Kruger *et al.*, 2002). There was no statistically significant difference between the men and women. There were significant differences ($p < 0.0001$) between men and women for all five skinfolds with women showing consistently thicker skinfolds than the men.

The median PAI-1_{act} level of the total group was 4.65 (IQR 2.10 - 7.80) IU/ml which was below the normal reference range of 8.3 - 12.4 IU/ml for healthy Caucasian individuals as given by the manufacturers of the test procedure (Spectrolyse[®] PAI-1 Activity Assay (Sekisui Diagnostics, LLC, Stamford, Connecticut, USA)). This reference range was used since a healthy range for Africans has not yet been determined and healthy reference ranges differ between kits due to a lack of standardisation between different companies. The median PAI-1_{act} levels of the men and women were 3.65 (IQR 0.99 - 6.93) IU/ml and 5.06 (IQR 2.73 – 8.35) IU/ml

respectively ($p < 0.0001$). Due to the difference in WC between men and women and the known relationship between PAI-1_{act} levels and WC, we adjusted for WC, but the significance remained. From the 2005 PURE data it was also reported that triglycerides correlated significantly ($r = 0.30$) with PAI-1_{act} and contributed to 5% of the variance in PAI-1_{act} levels (Pieters *et al.*, 2010). The median triglyceride level for the PURE study population in 2010 was 1.09 (IQR 0.82 – 1.55) mmol/L and the women had a significantly ($p = 0.041$) higher triglyceride level of 1.10 (IQR 0.85 – 1.63) mmol/L compared to the value for the men of 1.02 (IQR 0.76 – 1.42) mmol/L. Also, significantly higher PAI-1_{act} levels were observed in both men and women with hypertriglyceridemia (≥ 1.7 mmol/L) compared to participants with normal serum triglyceride values (data not shown). Therefore due to the gender difference in triglyceride level observed in this study and the relationship between triglyceride level and PAI-1_{act}, we adjusted for triglyceride level in addition to WC, but again the significant gender difference remained.

The median CLT for the total group was 62.2 (IQR 56.1 – 67.5) minutes whereas the median lysis times for men and women were 57.2 (IQR 51.6 – 62.3) minutes and 64.2 (IQR 59.6 – 69.4) minutes respectively ($p < 0.0001$). Similarly we adjusted for BMI due to the gender difference observed for BMI and the strong relationship between BMI and CLT, but again the significance remained. Clot lysis time was also found to be associated with triglyceride level in the 2005 data analysis (De Lange *et al.*, 2012) and in the present study CLT was found to be significantly longer in individuals with increased serum triglyceride levels ≥ 1.7 mmol/L (data not shown). We therefore additionally adjusted for triglyceride level and again the gender difference for CLT remained.

Table 4.1 Baseline characteristics of the South African PURE 2010 study participants

Variable	Total group (n= 1288)		Men (n= 435)		Women (n= 853)		p-value
	Median	25-75 Percentiles	Median	25-75 Percentiles	Median	25-75 Percentiles	
Age (yr)	53.7	47.2-61.4	54.2	47.1-62.0	53.4	47.3-61.0	0.42
PAI-1 _{act} (IU/ml)	4.65	2.10-7.80	3.65	0.99-6.93	5.06	2.73-8.35	<0.0001
*CLT (min)	62.2	56.1-67.5	57.2	51.6-62.3	64.2	59.6-69.4	<0.0001
Triglycerides (mmol/L)	1.09	0.82-1.55	1.02	0.76-1.42	1.10	0.85-1.63	0.041
BMI (kg/m ²)	24.0	19.8-30.2	20.2	18.1-23.1	27.0	22.1-32.8	<0.0001
Waist circumference (cm)	79.6	71.4-90.4	75.5	70.0-83.6	83.0	73.1-93.5	<0.0001
*Waist: height ratio	0.50	0.44-0.57	0.45	0.42-0.51	0.53	0.47-0.59	<0.0001
*WHR	0.88	0.83-0.93	0.94	0.88-0.98	0.86	0.81-0.90	<0.0001
Body fat (BP#) (%)	37.6	20.8-47.8	19.4	14.4-30.2	46.2	39.5-51.6	<0.0001
*Body fat (BIA#) (%)	32.9	19.7-41.5	27.9	15.6-38.7	34.9	22.0-42.6	<0.0001
Physical activity score	2.96	2.63-3.17	2.99	2.65-3.21	2.95	2.63-3.16	0.89
[€]Skinfolds:							
Chest (mm)	12.5	7.80-18.6	8.40	5.20-12.0	16.2	10.5-21.4	<0.0001
Abdomen (mm)	23.7	12.9-33.6	15.1	10.1-24.7	29.9	18.0-38.9	<0.0001
Thigh (mm)	23.2	9.80-43.3	8.60	5.90-13.0	36.6	22.0-51.2	<0.0001
Triceps (mm)	15.8	8.30-27.1	7.00	5.20-10.8	22.5	14.2-30.5	<0.0001
Supra iliac (mm)	13.9	8.20-23.9	8.85	5.40-12.6	19.8	11.8-27.4	<0.0001

PAI-1_{act}: plasminogen activator inhibitor type-1 activity; CLT: clot lysis time; BMI: body mass index; WHR: waist-to-hip ratio; BP: % body fat as determined with the BodPod® body compositions system; BIA: % body fat as determined by means of bioelectrical impedance analysis

*Variable had a normal distribution and therefore reported as mean (95% confidence interval (CI))

#BP data: total group n=144, men n=60, women n=84; BIA data: total group n=730, men n=234, women=496

[€]Skinfold data: chest: total group n=376, men n=139, women n=237; abdomen: total group n=376, men n=140, women n=236; thigh: total group n=374, men n=140, women n=234; triceps: total group n=1259, men n=425, women n=834; supra iliac: total group n=377, men n=140, women n=237

@p-value after adjustment for WC

¥p-value after adjustment for WC and triglycerides

^βp-value after adjustment for BMI

[€]p-value after adjustment for BMI and triglycerides

4.3 CORRELATIONS OF MARKERS OF FIBRINOLYSIS WITH DIFFERENT BODY COMPOSITION AND PHYSICAL ACTIVITY VARIABLES

4.3.1 Correlations of PAI-1_{act} with different body composition and physical activity variables

Table 4.2 reflects the Pearson correlations between PAI-1_{act} and different body composition and physical activity variables for men and women. Table 4.2 also reflects partial correlations between PAI-1_{act} and body composition and physical activity variables after adjustment for age, alcohol consumption, HIV status and smoking. Adjustment for these factors was needed since age, alcohol consumption, HIV status and smoking affected both PAI-1_{act} and anthropometric variables. In women, PAI-1_{act} correlated significantly ($p < 0.0001$) with BMI, WC, waist: height ratio and the triceps skinfold. The associations remained significant after the adjustments were made.

Compared to the women, PAI-1_{act} correlated not only significantly but also stronger with BMI ($p = 0.0003$ - for comparison of correlation coefficients between genders), WC ($p = 0.001$) and waist: height ratio ($p = 0.001$) in the men. In addition PAI-1_{act} also correlated significantly with the WHR and % body fat as measured with ADP. PAI-1_{act} also showed significant correlations with all the skinfolds in the men compared to a significant correlation only with the triceps skinfold in the women. All associations remained significant after adjustments were made. For the men, the correlation between PAI-1_{act} and TSF was significantly stronger ($p < 0.0001$) compared to the correlation in the women. PAI-1_{act} did not show any significant correlation with physical activity in either the women or the men.

Table 4.2 Correlations of PAI-1_{act} with different body composition and physical activity variables

Variable	Women					Men				
	n	Pearson correlations		*Partial correlations		n	Pearson correlations		*Partial correlations	
		r	p	r	p		r	p	r	p
BMI (kg/m ²)	843	0.23	<0.0001	0.14	<0.0001	781	0.30	<0.0001	0.31	<0.0001
Waist circumference (cm)	837	0.19	<0.0001	0.20	<0.0001	775	0.32	<0.0001	0.35	<0.0001
Waist: height ratio	837	0.19	<0.0001	0.20	<0.0001	775	0.31	<0.0001	0.35	<0.0001
WHR	819	0.06	0.11	0.06	0.10	758	0.17	0.001	0.19	<0.0001
Body fat (BP) (%)	84	-0.05	0.66	0.01	0.97	79	0.47	<0.0001	0.44	0.001
Body fat (BIA) (%)	496	0.07	0.11	0.08	0.08	449	-0.01	0.89	-0.01	0.92
Physical activity score	841	-0.03	0.32	-0.04	0.26	778	0.05	0.30	-0.00	0.96
<u>Skinfolds:</u>										
Chest (mm)	237	0.02	0.72	0.03	0.68	222	0.33	<0.0001	0.31	<0.0001
Abdomen (mm)	236	-0.01	0.92	0.01	0.91	221	0.36	<0.0001	0.38	<0.0001
Thigh (mm)	234	-0.02	0.72	0.00	0.10	219	0.34	<0.0001	0.30	0.001
Triceps (mm)	834	0.08	0.03	0.09	0.01	772	0.33	<0.0001	0.34	<0.0001
Supra iliac (mm)	237	0.08	0.26	0.09	0.21	222	0.35	<0.0001	0.31	<0.0001

BMI: body mass index; WHR: waist-to-hip ratio; BP: % body fat as determined using the BodPod® body compositions system; BIA: % body fat as determined by means of bioelectrical impedance analysis

*All variables were adjusted for age, alcohol consumption, HIV status and smoking

4.3.2 Correlations of CLT with different body composition and physical activity variables

Table 4.3 reflects the Pearson and partial correlations between CLT and various markers of body composition and physical activity for men and women. In the women, CLT correlated significantly with all the markers of body composition. Significant correlations were also seen between CLT and all the skinfolds. These significant correlations remained after adjustments were made for age, alcohol consumption, HIV status and smoking. Compared to PAI-1_{act}, CLT correlated significantly with more body composition variables in the women. In addition the correlations between CLT and BMI, WC, waist: height ratio and the triceps skinfold, were significantly stronger ($p < 0.0001$ for all four correlation comparisons) compared to the correlations between PAI-1_{act} and BMI, WC, waist: height ratio and the triceps skinfold in women.

In the men, CLT correlated significantly with all the skinfolds and most of the markers of body composition, except with % body fat as measured with BIA. These significant correlations also remained after the adjustments were made. The significant correlations between CLT and markers of body composition and the skinfolds in the men are similar in magnitude compared to the correlations between CLT and markers of body composition and the skinfolds in the women. However, WHR was an exception, where the correlation with CLT in the men was of stronger significance ($p = 0.009$) compared to the correlation with CLT in the women. Significant correlations of the same magnitude existed between CLT and PAI-1_{act} for the same variables in the men. One exception was the correlation with BMI. The correlation between CLT and BMI was significantly stronger ($p = 0.01$) compared to the correlation between PAI-1_{act} and BMI in the men. CLT did not show any significant correlation with physical activity in either the women or the men.

Table 4.3 Correlations of CLT with different body composition and physical activity variables

Variable	Women					Men				
	n	Pearson correlations		*Partial correlations		n	Pearson correlations		*Partial correlations	
		r	p	r	p		r	p	r	p
BMI (kg/m ²)	843	0.40	<0.0001	0.37	<0.0001	781	0.40	<0.0001	0.42	<0.0001
Waist circumference (cm)	837	0.42	<0.0001	0.40	<0.0001	775	0.37	<0.0001	0.40	<0.0001
Waist: height ratio	837	0.41	<0.0001	0.40	<0.0001	775	0.37	<0.0001	0.41	<0.0001
WHR	819	0.07	0.05	0.08	0.03	758	0.20	<0.0001	0.21	<0.0001
Body fat (BP) (%)	84	0.28	0.01	0.27	0.02	79	0.35	0.01	0.34	0.02
Body fat (BIA) (%)	496	0.10	0.03	0.12	0.01	449	0.08	0.26	0.07	0.31
Physical activity score	841	-0.03	0.43	-0.02	0.56	778	0.03	0.50	-0.02	0.68
<u>Skinfolds:</u>										
Chest (mm)	237	0.30	<0.0001	0.30	<0.0001	222	0.37	<0.0001	0.37	<0.0001
Abdomen (mm)	236	0.33	<0.0001	0.32	<0.0001	221	0.39	<0.0001	0.44	<0.0001
Thigh (mm)	234	0.23	<0.0001	0.23	0.001	219	0.40	<0.0001	0.38	<0.0001
Triceps (mm)	834	0.33	<0.0001	0.32	<0.0001	772	0.29	<0.0001	0.31	<0.0001
Supra iliac (mm)	237	0.38	<0.0001	0.38	<0.0001	222	0.40	<0.0001	0.38	<0.0001

BMI: body mass index; WHR: waist-to-hip ratio; BP: % body fat as determined with using the BodPod® body compositions system; BIA: % body fat as determined by means of bioelectrical impedance analysis

*All variables were adjusted for age, alcohol consumption, HIV status and smoking

4.4 DIFFERENCES IN MARKERS OF FIBRINOLYSIS BETWEEN TERILES AND CATEGORIES OF DIFFERENT BODY COMPOSITION VARIABLES

4.4.1 Differences in PAI-1_{act} between tertiles and categories of different body composition variables for men and women separately

The differences in mean PAI-1_{act} levels between tertiles and categories of different body composition variables are presented in Table 4.4 for the women and in Table 4.5 for the men. All non-parametric variables were log transformed to improve normality to allow use of parametric statistical tests. Least square means are reported in Tables 4.4 and 4.5. In the women, significant differences in mean PAI-1_{act} levels were observed between tertiles and categories of several anthropometric variables. The significant differences observed remained after adjustments were made for age, alcohol consumption, HIV status and smoking using ANCOVA tests.

PAI-1_{act} differed significantly between the underweight BMI category ($< 18.5 \text{ kg/m}^2$) and the other BMI categories, with PAI-1_{act} levels increasing as BMI increased. Subjects with a WC below the cut-off point suggested for African women (Crowther & Norris, 2012; Motala *et al.*, 2011), showed a lower mean PAI-1_{act} level compared to women with a WC that indicates central obesity (4.31 vs. 5.56 IU/ml, $p = 0.0018$). When using international cut-off points for abnormal waist circumferences (Alberti *et al.*, 2009; Alberti *et al.*, 2006; Grundy *et al.*, 2005), the same picture was observed. With the waist: height ratio a similar scenario as that of WC, was observed in the women. Women with a waist: height ratio indicating central obesity had higher PAI-1_{act} levels (5.21 vs. 3.77 IU/ml, $p = 0.0002$) compared to the women with a waist: height ratio not indicating central obesity. Differences in mean PAI-1_{act} levels between WHR tertiles were not significant ($p = 0.29$).

The difference in mean PAI-1_{act} levels between % body fat tertile 2 (as measured with ADP) and tertile 3 was borderline significant ($p = 0.053$). The strength of this significance increased to 0.035 after adjusting for age, alcohol consumption, HIV status and smoking. The significance of differences observed remained the same also after a Kruskal-Wallis ANOVA non-parametric test was performed for % body

Table 4.4 Differences in PAI-1_{act} between tertiles and categories of different body composition variables in women

Variable	Tertiles / categories		n	PAI-1 _{act} (IU/ml)		ANOVA p-value	ANCOVA p-value
				Mean ^a	CI		
BMI (kg/m ²)	Underweight	<18.5	75	2.79* ^{^#}	(2.15 - 3.56)	<0.0001	<0.0001
	Normal weight	≥18.5-<25.0	253	4.34*	(3.82 - 4.93)		
	Overweight	≥25.0-<30.0	201	5.13 [^]	(4.47 - 5.87)		
	Obese	≥30.0	292	5.17 [#]	(4.61 - 5.79)		
Waist circumference (cm) (using international cut-offs)	Normal WC	<80.0	350	3.80*	(3.40 - 4.23)	<0.0001	<0.0001
	Central obesity	≥80.0	465	5.38*	(4.92 - 5.88)		
Waist circumference (cm) (using cut-offs suggested for Africans)	Normal WC	<92.0	586	4.31*	(3.97 - 4.68)	0.0018	0.0015
	Central obesity	≥92.0	229	5.56*	(4.88 - 6.31)		
Waist: height ratio	Not centrally obese	<0.50	295	3.77*	(3.33 - 4.24)	0.00024	<0.0001
	Central obesity	≥0.50	520	5.21*	(4.78 - 5.67)		
WHR	Tertile 1	<0.83	275	4.45	(3.94 - 5.00)	0.29	0.30
	Tertile 2	≥0.83-<0.89	278	4.54	(4.03 - 5.11)		
	Tertile 3	≥0.89	245	5.06	(4.46 - 5.74)		
Body fat (BP) (%)	Tertile 1	<41.2	25	3.63	(1.97 - 6.22)	0.053 0.053(KW)	0.035
	Tertile 2	≥41.2-<49.3	28	5.82*	(3.51 - 9.34)		
	Tertile 3	≥49.3	29	2.11*	(1.06 - 3.71)		
Body fat (BIA) (%)	Tertile 1	<26.7	184	4.39	(3.83 - 5.03)	0.60	0.32
	Tertile 2	≥26.7-<40.1	187	4.95	(4.31 - 5.66)		
	Tertile 3	≥40.1	194	5.03	(4.40 - 5.74)		
Skinfolds:							
Chest (mm)	Tertile 1	<13.8	77	4.69	(3.55 - 6.12)	0.75	0.91
	Tertile 2	≥13.8-<24.7	76	4.30	(3.25 - 5.62)		
	Tertile 3	≥24.7	76	4.54	(3.40 - 5.98)		
Abdomen (mm)	Tertile 1	<21.9	77	5.02	(3.83 - 6.51)	0.49	0.65
	Tertile 2	≥21.9-<34.6	75	4.45	(3.36 - 5.83)		
	Tertile 3	≥34.6	77	4.19	(3.11 - 5.54)		
Thigh (mm)	Tertile 1	<26.6	76	5.39*	(4.10 - 7.00)	0.043	0.075
	Tertile 2	≥26.6-<46.4	76	3.53*	(2.62 - 4.66)		
	Tertile 3	≥46.4	74	5.00	(3.77 - 6.56)		
Triceps (mm)	Tertile 1	<16.9	269	4.29	(3.77 - 4.87)	0.20	0.14
	Tertile 2	≥16.9-<28.1	269	4.52	(4.00 - 5.10)		
	Tertile 3	≥28.1	274	5.13	(4.54 - 5.79)		
Supra iliac (mm)	Tertile 1	<13.8	76	4.14	(3.10 - 5.43)	0.84	0.69
	Tertile 2	≥13.8-<24.7	77	4.89	(3.72 - 6.34)		
	Tertile 3	≥24.7	76	4.53	(3.38 - 5.97)		

PAI-1_{act}: plasminogen activator inhibitor type-1 activity; BMI: body mass index; WHR: waist-to-hip ratio; BP: % body fat as determined with using the BodPod® body compositions system; BIA: % body fat as determined by means of bioelectrical impedance analysis; KW: Kruskal-Wallis ANOVA

^a Least square means obtained from ANCOVA reported

*^{^#} Means with the same symbol differed significantly between tertiles / categories; significance at $p < 0.05$

ANCOVA adjusted for age, alcohol consumption, HIV status and smoking

Table 4.5 Differences in PAI-1_{act} between tertiles and categories of different body composition variables in men

Variable	Tertiles / categories		n	PAI-1 _{act} (IU/ml)		ANOVA p-value	ANCOVA p-value
				Mean ^a	CI		
BMI (kg/m ²)	Underweight	<18.5	123	2.04 ^{*A#}	(1.57 - 2.60)	0.0002	0.00019
	Normal weight	≥18.5-<25.0	230	3.33 [*]	(2.83 - 3.89)		
	Overweight	≥25.0-<30.0	51	4.32 ^A	(3.10 - 5.89)		
	Obese	≥30.0	21	5.68 [#]	(3.25 - 9.49)		
Waist circumference (cm) (using international cut-offs)	Normal WC	<94.0	382	2.84 [*]	(2.50 - 3.21)	<0.0001	<0.0001
	Central obesity	≥94.0	39	6.44 [*]	(4.51 - 9.06)		
Waist circumference (cm) (using cut-offs suggested for Africans)	Normal WC	<86.0	334	2.64 [*]	(2.30 - 3.03)	<0.0001	<0.0001
	Central obesity	≥86.0	87	5.25 [*]	(4.10 - 6.65)		
Waist: height ratio	Not centrally obese	<0.50	308	2.51 [*]	(3.16 - 3.89)	<0.0001	<0.0001
	Central obesity	≥0.50	113	5.13 [*]	(4.14 - 6.30)		
WHR	Tertile 1	<0.90	141	2.01 ^{*A}	(1.58 - 2.51)	0.00026	<0.0001
	Tertile 2	≥0.90-<0.97	135	4.05 [*]	(3.32 - 4.91)		
	Tertile 3	≥0.97	134	3.30 ^A	(2.67 - 4.04)		
Body fat (BP) (%)	Tertile 1	<15.7	18	0.92 [*]	(0.13 - 2.29)	0.0013 0.0011(KW)	0.0038
	Tertile 2	≥15.7-<24.0	20	1.52 [‡]	(0.51 - 3.20)		
	Tertile 3	≥24.0	20	5.85 ^{*‡}	(3.12 - 10.41)		
Body fat (BIA) (%)	Tertile 1	<19.6	91	2.93	(2.25 - 3.77)	0.33	0.47
	Tertile 2	≥19.6-<35.1	99	3.58	(2.81 - 4.49)		
	Tertile 3	≥35.1	99	3.52	(2.75 - 4.45)		
Skinfolds:							
Chest (mm)	Tertile 1	<5.80	43	2.11 [*]	(1.25 - 3.30)	0.018	0.027
	Tertile 2	≥5.80-<10.4	46	2.57	(1.61 - 3.87)		
	Tertile 3	≥10.4	48	4.62 [*]	(3.14 - 6.64)		
Abdomen (mm)	Tertile 1	<11.2	44	1.32 [£]	(0.71 - 2.14)	<0.0001	<0.0001
	Tertile 2	≥11.2-<19.1	49	3.03 ^{£A}	(2.05 - 4.33)		
	Tertile 3	≥19.1	45	5.64 ^{£A}	(3.93 - 7.95)		
Thigh (mm)	Tertile 1	<6.60	46	2.10 [*]	(1.29 - 3.20)	0.0013	0.0087
	Tertile 2	≥6.60-<11.0	45	2.44 ^A	(1.52 - 3.70)		
	Tertile 3	≥11.0	47	5.05 ^{*A}	(3.42 - 7.28)		
Triceps (mm)	Tertile 1	<5.70	136	1.83 [*]	(1.42 - 2.30)	<0.0001	<0.0001
	Tertile 2	≥5.70-<9.20	137	3.12 [*]	(2.55 - 3.79)		
	Tertile 3	≥9.20	131	4.71 [*]	(3.85 - 5.72)		
Supra iliac (mm)	Tertile 1	<6.20	45	1.78 [*]	1.04 - 2.79)	0.0025	0.014
	Tertile 2	≥6.20-<11.2	47	3.32	(2.15 - 4.92)		
	Tertile 3	≥11.2	46	4.37 [*]	(2.93 - 6.32)		

PAI-1_{act}: plasminogen activator inhibitor type-1 activity; BMI: body mass index; WHR: waist-to-hip ratio; BP: % body fat as determined with using the BodPod® body compositions system; BIA: % body fat as determined by means of bioelectrical impedance analysis; KW: Kruskal-Wallis ANOVA

^a Least square means obtained from ANCOVA reported

^{*A#} Means with the same symbol differed significantly between tertiles / categories; significance at p < 0.05

[‡] Means with the same symbol differed significantly between tertiles / categories; significance at p < 0.05 using ANCOVA; this in addition to the differences observed with ANOVA

[£] Means with the same symbol differed significantly between tertiles / categories; significance at p < 0.05 using ANOVA and ANCOVA

ANCOVA adjusted for age, alcohol consumption, HIV status and smoking

fat as measured with ADP. We wanted to confirm the results using a non-parametric test, due to the smaller sample size available for these variables.

When % body fat was measured with BIA, mean PAI-1_{act} levels did not show any significant difference ($p = 0.60$) between % body fat tertiles. The thigh skinfold was the only skinfold in the women where a significant difference in the PAI-1_{act} level was observed between tertile 1 and tertile 2 ($p = 0.043$).

In the men, significant differences in mean PAI-1_{act} levels were also observed between tertiles and categories of several anthropometric variables. A picture similar to that seen in the women was observed for differences in mean PAI-1_{act} levels between the different BMI categories. There were significant differences ($p = 0.0002$) between the underweight ($< 18.5 \text{ kg/m}^2$) and the other BMI categories.

Irrespective of using international cut-off points for abnormal waist circumferences (Alberti *et al.*, 2009; Alberti *et al.*, 2006; Grundy *et al.*, 2005) or cut-off points suggested for African men (Crowther & Norris, 2012; Motala *et al.*, 2011), the picture remained the same, with lower PAI-1_{act} levels in the groups below the central obesity cut-offs. For the men, the waist: height ratio showed a similar picture to what was seen in the waist: height ratio of the women. The category indicating central obesity had higher PAI-1_{act} levels compared to the category not indicating central obesity (5.13 vs. 2.51 IU/ml, $p < 0.0001$).

PAI-1_{act} levels increased significantly ($p = 0.001$) as % body fat (as measured with ADP) increased. This was similar to what was observed in women. Tertile 1 showed a mean PAI-1_{act} level of 0.92 IU/ml with an increasing mean PAI-1_{act} level of 5.85 IU/ml in % body fat tertile 3. The significance of this difference remained after a Kruskal-Wallis ANOVA non-parametric test was performed (to account for the smaller sample size). However, the ANCOVA test showed a significant difference between tertile 2 and tertile 3, in addition to the significant difference between tertile 1 and tertile 3.

No significant differences were seen in mean PAI-1_{act} levels across % body fat tertiles (as measured with BIA) ($p = 0.33$) in either men or women. For all the

skinfolts, mean PAI-1_{act} levels increased as the thickness of the skinfolts increased across tertiles. This is different to what was observed in women where PAI-1_{act} increased only across thigh skinfold categories.

4.4.2 Differences in CLT between tertiles and categories of different body composition variables for men and women separately

The differences in mean CLT between tertiles and categories of different body composition variables are presented in Table 4.6 for the women and in Table 4.7 for the men. Significant differences in mean CLTs were observed in the women between the tertiles and categories of several anthropometric variables as well as all the skinfolts. ANCOVA tests were again used to adjust for age, alcohol consumption, HIV status and smoking. Again the significant differences observed remained after adjustments were done. For CLT an additional adjustment was made for PAI-1_{act} in order to determine whether the observed association between CLT and body composition was in fact a reflection of the relationship between PAI-1_{act} and body composition with PAI-1_{act} being a significant predictor of CLT. Adjustment for PAI-1_{act} did not, however, alter the significance, indicating that CLT is associated with body composition, at least in part independently of PAI-1_{act}.

Mean CLTs increased significantly ($p < 0.0001$) as the BMI category increased in the women. A mean CLT of 58.8 min were seen in the underweight category and CLT increased to 68.9 min in the obese category. A significant increase in mean CLT was seen as the WC and waist: height ratio of the women increased. There was a significant difference between the two WC categories, as suggested for African women (Crowther & Norris, 2012; Motala *et al.*, 2011), (63.5 vs. 69.1 min, $p < 0.0001$). When international cut-off points for abnormal waist circumferences (Alberti *et al.*, 2009; Alberti *et al.*, 2006; Grundy *et al.*, 2005) were used, the same picture emerged.

Table 4.6 Differences in CLT between tertiles and categories of different body composition variables in women

Variable	Tertiles / categories		n	CLT (min)		ANOVA p-value	ANCOVA p-value
				Mean ^a	CI		
BMI (kg/m ²)	Underweight	<18.5	75	58.8*	(56.9 - 60.7)	<0.0001	<0.0001 (<0.0001)
	Normal weight	≥18.5-<25.0	251	61.9*	(60.8 - 63.0)		
	Overweight	≥25.0-<30.0	196	65.8*	(64.6 - 67.0)		
	Obese	≥30.0	287	68.9*	(67.9 - 69.9)		
Waist circumference (cm) (using international cut-offs)	Normal WC	<80.0	350	61.3*	(60.4 - 62.2)	<0.0001	<0.0001 (<0.0001)
	Central obesity	≥80.0	453	68.0*	(67.2 - 68.8)		
Waist circumference (cm) (using cut-offs suggested for Africans)	Normal WC	<92.0	581	63.5*	(62.8 - 64.2)	<0.0001	<0.0001 (<0.0001)
	Central obesity	≥92.0	222	69.1*	(67.9 - 70.3)		
Waist: height ratio	Not centrally obese	<0.50	294	60.7*	(59.7 - 61.7)	<0.0001	<0.0001 (<0.0001)
	Central obesity	≥0.50	509	67.6*	(66.9 - 68.4)		
WHR	Tertile 1	<0.83	274	64.2	(63.2 - 65.3)	0.19	0.13 (0.25)
	Tertile 2	≥0.83-<0.89	273	65.3	(64.2 - 66.4)		
	Tertile 3	≥0.89	247	65.8	(64.7 - 67.0)		
Body fat (BP) (%)	Tertile 1	<41.2	24	63.8	(59.9 - 67.7)	0.15 0.14(KW)	0.14 (0.29)
	Tertile 2	≥41.2-<49.3	27	69.0	(65.4 - 72.7)		
	Tertile 3	≥49.3	28	65.2	(61.6 - 68.9)		
Body fat (BIA) (%)	Tertile 1	<26.7	182	64.3*	(63.0 - 65.5)	0.0048	0.0024 (0.006)
	Tertile 2	≥26.7-<40.1	182	66.4	(65.1 - 67.7)		
	Tertile 3	≥40.1	192	67.4*	(66.1 - 68.7)		
Skinfolds:							
Chest (mm)	Tertile 1	<13.8	76	60.8* [^]	(58.8 - 62.8)	<0.0001	<0.0001 (<0.0001)
	Tertile 2	≥13.8-<24.7	75	65.3*	(63.4 - 67.2)		
	Tertile 3	≥24.7	71	67.8 [^]	(65.8 - 69.8)		
Abdomen (mm)	Tertile 1	<21.9	76	61.0* [^]	(59.0 - 62.9)	<0.0001	<0.0001 (<0.0001)
	Tertile 2	≥21.9-<34.6	73	65.8*	(63.8 - 67.8)		
	Tertile 3	≥34.6	76	67.2 [^]	(65.2 - 69.3)		
Thigh (mm)	Tertile 1	<26.6	75	62.5*	(60.5 - 64.6)	0.0056	0.0075 (0.002)
	Tertile 2	≥26.6-<46.4	71	64.1	(62.1 - 66.1)		
	Tertile 3	≥46.4	73	67.3*	(65.2 - 69.4)		
Triceps (mm)	Tertile 1	<16.9	267	61.7*	(60.6 - 62.8)	<0.0001	<0.0001 (<0.0001)
	Tertile 2	≥16.9-<28.1	264	64.9*	(63.9 - 66.0)		
	Tertile 3	≥28.1	269	68.5*	(67.4 - 69.6)		
Supra iliac (mm)	Tertile 1	<13.8	75	61.0* [^]	(59.0 - 63.0)	<0.0001	<0.0001 (<0.0001)
	Tertile 2	≥13.8-<24.7	76	64.5*	(62.6 - 66.5)		
	Tertile 3	≥24.7	71	68.3 [^]	(66.3 - 70.3)		

CLT: Clot lysis time; BMI: body mass index; WHR: waist-to-hip ratio; BP: % body fat as determined with using the BodPod® body compositions system; BIA: % body fat as determined by means of bioelectrical impedance analysis; KW: Kruskal-Wallis ANOVA

^a Least square means obtained from ANCOVA reported

*[^] Means with the same symbol differed significantly between tertiles / categories; significance at $p < 0.05$

ANCOVA: adjusted for age, alcohol consumption, HIV status and smoking

p-value in brackets: p-value after additional adjustment for PAI-1_{act}

Table 4.7 Differences in CLT between tertiles and categories of different body composition variables in men

Variable	Tertiles / categories		n	CLT (min)		ANOVA p-value	ANCOVA p-value
				Mean ^a	CI		
BMI (kg/m ²)	Underweight	<18.5	123	53.9*	(52.3 - 55.4)	<0.0001	<0.0001 (<0.0001)
	Normal weight	≥18.5-<25.0	224	57.2* [^]	(56.1 - 58.3)		
	Overweight	≥25.0-<30.0	50	61.5* [^]	(59.2 - 63.9)		
	Obese	≥30.0	19	65.8* [^]	(61.7 - 69.9)		
Waist circumference (cm) (using international cut-offs)	Normal WC	<94.0	374	56.4*	(55.6 - 57.3)	<0.0001	<0.0001 (<0.0001)
	Central obesity	≥94.0	38	65.0*	(62.2 - 67.8)		
Waist circumference (cm) (using cut-offs suggested for Africans)	Normal WC	<86.0	328	55.9*	(55.0 - 56.9)	<0.0001	<0.0001 (<0.0001)
	Central obesity	≥86.0	84	62.0*	(60.1 - 63.9)		
Waist: height ratio	Not centrally obese	<0.50	304	55.5*	(54.5 - 56.4)	<0.0001	<0.0001 (<0.0001)
	Central obesity	≥0.50	108	61.9*	(60.3 - 63.5)		
WHR	Tertile 1	<0.90	140	54.7* [^]	(53.3 - 56.2)	0.00014	<0.0001 (0.009)
	Tertile 2	≥0.90-<0.97	130	59.3*	(57.8 - 60.8)		
	Tertile 3	≥0.97	131	57.6 [^]	(56.1 - 59.1)		
Body fat (BP) (%)	Tertile 1	<15.7	18	56.1*	(52.2 - 59.9)	<0.0001 <0.0001(KW)	<0.0001 (<0.0001)
	Tertile 2	≥15.7-<24.0	19	48.5 [^]	(44.9 - 52.2)		
	Tertile 3	≥24.0	20	65.7* [^]	(62.1 - 69.3)		
Body fat (BIA) (%)	Tertile 1	<19.6	89	57.0	(55.2 - 58.9)	0.07	0.064 (0.06)
	Tertile 2	≥19.6-<35.1	95	56.5 [¥]	(54.7 - 58.2)		
	Tertile 3	≥35.1	96	59.3 [¥]	(57.5 - 61.2)		
Skinfolds:							
Chest (mm)	Tertile 1	<5.80	43	52.9*	(50.1 - 55.7)	0.004	0.0028 (0.04)
	Tertile 2	≥5.80-<10.4	46	55.1 [¥]	(52.4 - 57.8)		
	Tertile 3	≥10.4	48	59.7* [¥]	(57.0 - 62.3)		
Abdomen (mm)	Tertile 1	<11.2	44	51.9*	(49.3 - 54.5)	<0.0001	<0.0001 (0.0002)
	Tertile 2	≥11.2-<19.1	49	53.9 [^]	(51.5 - 56.3)		
	Tertile 3	≥19.1	45	62.2* [^]	(59.7 - 64.8)		
Thigh (mm)	Tertile 1	<6.60	46	52.9*	(50.3 - 55.6)	0.00050	0.0018 (0.04)
	Tertile 2	≥6.60-<11.0	45	55.2 [¥]	(52.4 - 57.9)		
	Tertile 3	≥11.0	47	60.0* [¥]	(57.3 - 62.8)		
Triceps (mm)	Tertile 1	<5.70	136	54.3*	(52.8 - 55.8)	<0.0001	<0.0001 (0.001)
	Tertile 2	≥5.70-<9.20	142	56.8 [^]	(55.4 - 58.3)		
	Tertile 3	≥9.20	131	60.3* [^]	(58.8 - 61.9)		
Supra iliac (mm)	Tertile 1	<6.20	45	52.2*	(49.5 - 54.9)	0.0007	0.0021 (0.04)
	Tertile 2	≥6.20-<11.2	47	56.5	(53.8 - 59.3)		
	Tertile 3	≥11.2	46	59.2*	(56.5 - 61.9)		

CLT: Clot lysis time; BMI: body mass index; WHR: waist-to-hip ratio; BP: % body fat as determined with using the BodPod® body compositions system; BIA:

% body fat as determined by means of bioelectrical impedance analysis; KW: Kruskal-Wallis ANOVA

^a Least square means obtained from ANCOVA reported

*[^] Means with the same symbol differed significantly between tertiles / categories; significance at $p < 0.05$

[¥] Means with the same symbol differed significantly between tertiles / categories; significance at $p < 0.05$ using ANCOVA; this in addition to the differences observed with ANOVA

ANCOVA: adjusted for age, alcohol consumption, HIV status and smoking

p-value in brackets: p-value after additional adjustment for PAI-1_{act}

The waist: height ratio category < 0.50 had a mean CLT of 60.7 min whereas the category indicative of obesity had a higher mean CLT of 67.6 min ($p < 0.0001$). No significant differences for the women were seen in mean CLTs between the tertiles for the WHR ($p = 0.19$), and % body fat ($p = 0.15$) as measured with ADP. This remained the same when a Kruskal-Wallis ANOVA non-parametric test was performed. Clot lysis time increased significantly across the % body fat tertiles as measured with BIA.

Mean CLTs increased for all the skinfolds as the thickness of the skinfolds increased. The association of CLT with skinfolds showed a very different picture compared to the association of PAI-1_{act} with skinfolds in the women.

For the men significant differences in mean CLTs were observed between the tertiles and categories of most of the body composition variables. ANCOVA tests were again done to adjust for age, alcohol consumption, HIV status and smoking. The significant differences observed remained after adjustments were made. Significance also remained after adjustment for PAI-1_{act}.

In the men, similar to the women, mean CLTs increased as the BMI category increased. The picture for WC is also the same for the men as that for the women. A significant increase ($p < 0.0001$) was seen in mean CLT as the WC of the men increased using both the African as well as the international cut-offs. The picture for the waist: height ratio in the men is very similar to that of the WC in the men. Compared to no significant difference for the women in mean CLTs between the WHR tertiles, significant differences ($p = 0.0001$) were observed between the tertiles for the men. Mean CLT increased from tertile 1 to tertile 2, but then decreased in tertile 3.

The % body fat as measured with ADP showed significant differences ($p < 0.0001$) in mean CLT between tertiles, with the lowest PAI-1_{act} levels observed in tertile 2. This is in contrast with no differences found in mean CLT between the % body fat tertiles in the women. Significant differences observed remained the same when a Kruskal-Wallis ANOVA non-parametric test was performed. CLT tended to increase across % body fat categories as measured with BIA. Mean CLTs increased for all the

skinfolts as the thickness of the skinfolts of the men increased. This picture is the same as seen for the mean CLTs across the skinfold tertiles of the women.

4.4.3 Comparison of the association of PAI-1_{act} and CLT with the body composition markers in women

In the women the mean PAI-1_{act} levels and CLTs increased significantly over BMI, WC, and waist: height ratio categories. For BMI, PAI-1_{act} levels seem to reach a plateau at the highest BMI category while a significant increase is observed for CLT. Mean PAI-1_{act} levels showed an initial increase as % body fat (as measured with ADP) increased but thereafter the PAI-1_{act} level decreased as % body fat continued to increase. A similar trend was seen with changes in mean CLTs across % body fat tertiles when compared to changes in mean PAI-1_{act} levels, except that the differences between categories were not significant.

For % body fat (as measured with BIA), mean PAI-1_{act} levels showed a non-significant trend to increase as % body fat increased while mean CLT increased significantly as % body fat increased.

For the skinfolts, differences in mean PAI-1_{act} levels were very small and non-significant, except for a small difference across the thigh skinfold tertiles. Bigger and statistically significant differences were, however, observed in mean CLTs across tertiles of all the skinfolts, with longer CLTs as the skinfolts increased.

4.4.4 Comparison of the association of PAI-1_{act} and CLT with the body composition markers in men

Mean PAI-1_{act} levels and CLT in the men also increased significantly over BMI, WC, and waist: height ratio categories, as well as the WHR tertiles. In men, PAI-1_{act} levels did not plateau at the higher BMI categories as was seen in women but continued to increase similarly to the increase in CLT. Mean PAI-1_{act} levels showed significant increases as % body fat (as measured with ADP) increased. Mean CLTs, on the other hand, differed across the body fat tertiles, with decreased levels observed in tertile 2 before CLTs increased significantly in the highest tertile.

For % body fat (as measured with BIA), no differences were observed for mean PAI-1_{act} levels across tertiles. In contrast to this, mean CLT increased with borderline significance across tertiles.

For the skinfolds of the men, a similar picture is seen for both PAI-1_{act} and CLT. Mean PAI-1_{act} levels and mean CLTs differed significantly between tertiles of all skinfolds, with higher PAI-1_{act} levels and longer CLTs observed as the skinfolds increased.

In order to illustrate the differences in the association of PAI-1_{act} and CLT with body composition markers, within as well as between genders, the following graphs were constructed. Figures 1 and 2 depict the increase in PAI-1_{act} and CLT across BMI categories and triceps skinfolds for men and women separately.

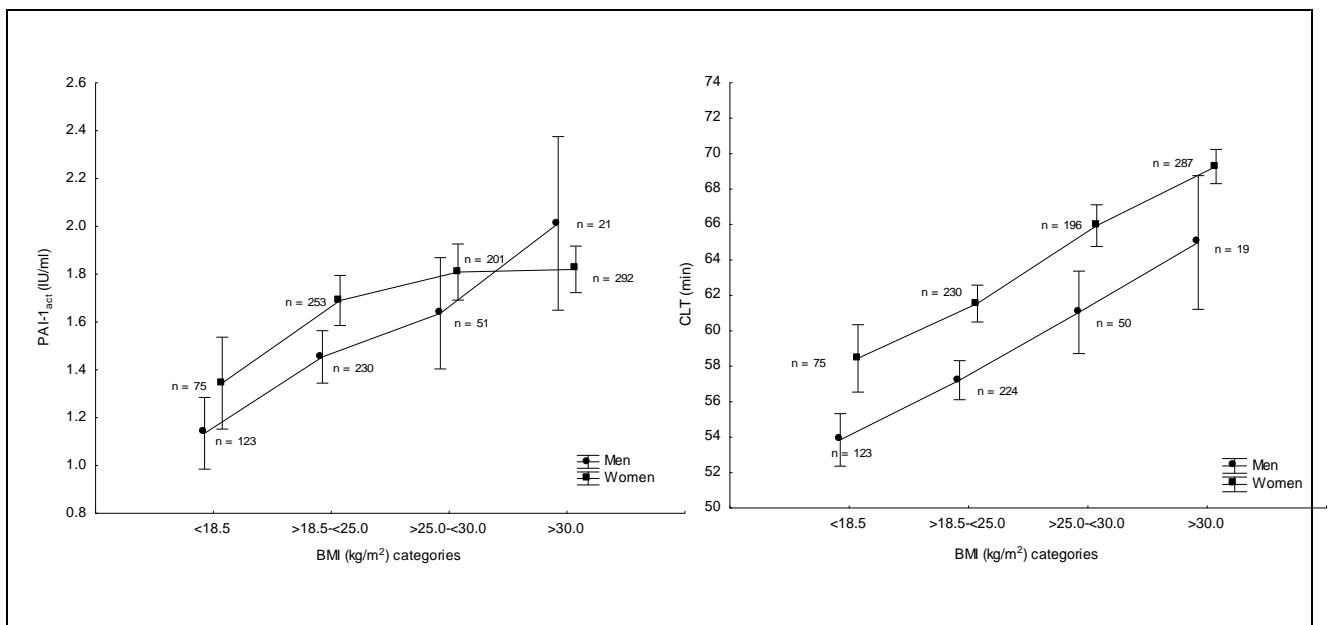


Figure 4.1: The association of PAI-1_{act} and CLT with BMI categories within as well as between genders

From Figure 1 it is clear that there is a discordance between the association of PAI-1_{act} with BMI and the association of CLT with BMI in women, while in men both increase across BMI categories. In women PAI-1_{act} plateaus at the higher BMI categories, while CLT continues to increase.

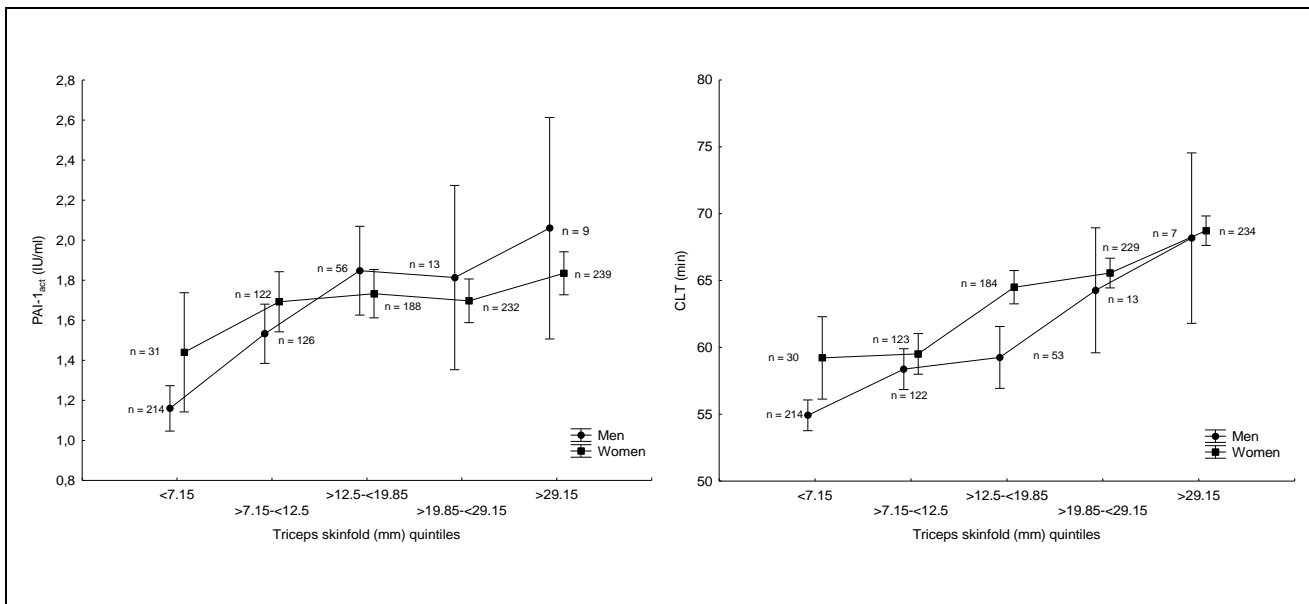


Figure 4.2: The association of PAI-1_{act} and CLT with triceps skinfold quintiles within as well as between genders

Gender differences can also be observed for associations with triceps skinfold. In women there is a much weaker association ($r = 0.055$) between PAI-1_{act} and triceps skinfold compared to the men ($r = 0.23$), while for CLT there is a general increase across triceps quintiles in both genders.

The results will now be discussed in detail in Chapter 5.

CHAPTER 5: DISCUSSION AND CONCLUSION

5.1 INTRODUCTION

The main focus of this study was to investigate the association of markers of fibrinolysis, PAI-1_{act} level and CLT, with a variety of body composition variables and physical activity, in the PURE 2010 study population. This chapter therefore briefly describes the anthropometric and fibrinolytic profiles of the men and women in the study population. This is followed by a discussion of the association of PAI-1_{act} and CLT with markers of body composition. Differences in the association of PAI-1_{act} and CLT with body composition markers will receive special attention, as well as the role of gender.

5.2 PARTICIPANT CHARACTERISTICS

5.2.1 Anthropometric profile of the PURE 2010 study population

The South African National Health and Nutrition Examination Survey (SANHANES) reported that in the South African population overweight and obesity were significantly higher in the women (64.0%) than in the men (30.7%) (Shisana *et al.*, 2013). Similar pictures were seen between women and men living in the North-West Province and between black African men and women. In the 2010 PURE study population the women presented with a comparatively higher mean BMI than the men with more women (47.6%) than men (29.4%) being overweight and obese (BMI exceeding 25 kg/m²). Another similar picture was found in the well-documented THUSA study in the North-West Province in 1996 (Vorster *et al.*, 2000; Vorster 2002; Vorster *et al.*, 2005; Vorster *et al.*, 2007; Kruger *et al.*, 2012). Very few men were overweight or obese (13.4%) whereas more than half of the women (53.8%) had a BMI that exceeded 25 kg/m² (van Rooyen *et al.*, 2000; Vorster *et al.*, 2000; Kruger *et al.*, 2002; Kruger *et al.*, 2003). Kruger & co-workers (2012) concluded that overweight/obesity in the women of this study population was strongly and significantly associated with the female sex. The same scenario was already

present in 2005 in the PURE study population where more men within the normal BMI range (De Lange *et al.*, 2012; Pieters *et al.*, 2011), and more women in the overweight and obese range (De Lange *et al.*, 2012; Pisa *et al.*, 2012) were reported.

In this study different measures of abdominal/central obesity were investigated, namely the WHR, WC and the waist: height ratio. Together with the BMI and skinfold measurements, these indices can be used to identify individuals with elevated obesity-related health risks (Katzmarzyk *et al.*, 2011; WHO, 2011d). It must however be kept in mind that not one of these indices or measurements can differentiate between abdominal subcutaneous adipose tissue (SAT) and abdominal visceral adipose tissue (VAT) (this will be discussed in greater detail in the next section), but can only indicate overall obesity (BMI) or general obesity in the central region (WHR, WC and waist: height ratio).

The female hormone estrogen favors the storage of peripheral adipose tissue, whereas the male hormone testosterone favors the increase in lean tissue mass and the storage of abdominal adipose tissue (Rosenbaum & Leibel, 1999). Since women have a tendency towards peripheral fat distribution in the gluteal-femoral region and men have a tendency towards visceral (abdominal) fat distribution (Bray *et al.*, 2008; Machann *et al.*, 2005; Garaulet *et al.*, 2000; Lemieux *et al.*, 1993), a higher WHR in men compared to women can be expected when excess body fat is accumulated. In addition, women may present with a higher WC as well as a higher hip circumference compared to men and therefore the WHR of the woman will be smaller than that of the man. This can be seen in the higher mean WHR of the men of 0.94 in the 2010 PURE study participants compared to that of the women of 0.86. The SANHANES reported a similar picture of a higher WHR for the men than the women (Shisana *et al.*, 2013). A WHR above recommended sex-specific cut-off points is associated with a risk of metabolic complications and indicates abdominal obesity. These cut-off points are ≤ 0.90 for men and ≤ 0.85 for women (WHO, 2011d). Compared to the cut-off points, the median WHRs of both the men and women in the present study were close to the cut-off point for abdominal obesity.

A mean WC at population level that exceeds the recommended WC, indicates a substantially increased risk of metabolic complications (Shisana *et al.*, 2013). Gender and ethnicity not only affect BMI, but also WC (Sumner, 2008). Mean WC for the present study for both genders is very similar to that reported for the PURE study in 2005 (de Lange *et al.*, 2012; Pieters *et al.*, 2011). Based on average WC, in this study, the women had a significantly higher WC than the men. More women (57.1%) than men (9.3%) presented with abdominal obesity when international recommended cut-off points for sub-Saharan Africans (Alberti *et al.*, 2009; Alberti *et al.*, 2006; Grundy *et al.*, 2005) were used. De Lange *et al.* (2012) reported an abdominal obesity prevalence of 34.6% for the total PURE group in 2005 and in 2001, Kruger *et al.* reported a central obesity rate of 39.1% among the women in the THUSA study. A similar picture was reported from the SANHANES (Shisana *et al.*, 2013) where more women (68.2%) than men (20.2%) in the total South African population presented with a WC that exceeded the sex-specific cut-off points. The same was seen for the population of the North-West Province (61.9% and 15.3% for women and men respectively) and for black Africans (67.6% and 17.4% for women and men respectively) (Shisana *et al.*, 2013). However, the same criteria for WC to diagnose central obesity cannot be applied to all populations and ethnic groups due to heterogeneity of abdominal tissue composition (Misra *et al.*, 2005). Therefore, when other WC cut-offs suggested to indicate central obesity in Africans (Crowther & Norris, 2012; Motala *et al.*, 2011) were used in this study, the picture changed somewhat to central obesity in 28.1% of the women and 20.7% in the men respectively. Yet, despite the use of different WC cut-offs, overall more women than men presented with central obesity in the 2010 PURE study population when using WC as criterion.

The waist: height ratio as an index of central obesity has been associated with risk factors for CVD and a universal cut-off value of ≥ 0.50 indicates central fat distribution and CVD risk (Freedman *et al.*, 2007; Parikh *et al.*, 2007; Hsieh *et al.*, 2003). Based on the waist: height ratio, the prevalence of central obesity among women in this study (63.8%) was higher compared to that of the men (26.8%). Similar results were reported for this study population in 2005 (Beneke, 2009).

It is clear from both the PURE and THUSA studies that women presented with higher values for several markers of obesity compared to men. This is in accordance with other studies that also suggested that obesity in the black African population is generally more prevalent in women than in men (Akinboboye *et al.*, 2003). Therefore, although the overall picture is one of more women than men presenting with central obesity in this population, it must be remembered, as previously stated, that men and women differ with regard to adipose tissue type, distribution and sequence of adipose tissue accumulation.

Gender differences in adipose tissue distribution and type are well documented with visceral fat and subcutaneous fat being different regarding metabolic activity and histology (Garaulet *et al.*, 2006). At any specific BMI, a woman will present with a proportionally higher level of body fat and a man with a higher level of lean mass (Garaulet *et al.*, 2000). As already mentioned, men show a tendency towards visceral (abdominal) fat distribution and women have a tendency towards peripheral fat distribution in the gluteal-femoral region. Peripheral fat distribution is also referred to as the gynoid shape and central fat distribution as the android shape (Bray *et al.*, 2008; Machann *et al.*, 2005; Garaulet *et al.*, 2000; Lemieux *et al.*, 1993). Computerized tomography scans or magnetic resonance images revealed that, compared to women, men have in general less peripheral or SAT but more VAT and hepatic adipose tissue (Bray *et al.*, 2008; Machann *et al.*, 2005; Garaulet *et al.*, 2000; Kvist *et al.*, 1988). Therefore an obese man will have proportionally more VAT than an obese woman and an obese woman will have proportionally more SAT (both central and peripheral) than the obese man (Geer & Shen 2009). Central or abdominal fat is composed of both abdominal VAT and abdominal SAT (Wajchenberg, 2000). From the discussion earlier about the anthropometric profile of the 2010 PURE study participants, it is clear that the women in general have a higher WC and waist: height ratio compared to the men, indicating central obesity. However, even though WC is a good indicator of the total amount of abdominal fat, WC alone as a measurement cannot differentiate between abdominal VAT and abdominal SAT. Despite the higher WC in women they have a proportionally even larger hip circumference suggesting a gynoid fat distribution with a larger proportion of SAT in the periphery. Excess abdominal fat is well documented to be a cardiometabolic risk factor. Moreover, a poor cardiometabolic profile has been

demonstrated to correlate stronger with an excess of abdominal visceral fat compared to subcutaneous obesity which is considered to contribute much less to metabolic risk (Després *et al.*, 2008).

The above discussion can be further motivated by looking at the values of the skinfolds of the 2010 PURE study participants. The abdominal and supra iliac skinfolds represent SAT in the abdominal region. Both these skinfolds were twice the amount in the women than in the men of this study. The thigh and the triceps skinfolds represent SAT in the peripheral regions and these two skinfolds show even bigger differences between the men and women. In the women the triceps and the thigh skinfolds were three and four times the values of the men respectively. These findings support the observations mentioned earlier that the women in this study not only have more SAT than the men but also more fat distributed in the subcutaneous fat compartment of the peripheral regions. This is in spite of the fact that these women presented with a higher WC and waist: height ration than the men.

The VAT compartment of the body does not only differ between genders but is also affected by race and ethnicity (Conway *et al.*, 1995). This was illustrated by a study done in severely obese African-American and Caucasian women. This study reported that the African-American women had smaller amounts of VAT, and therefore higher amounts of SAT than the Caucasian women, despite similar BMIs, WHRs and waist circumferences (Conway *et al.*, 1995). In 1997 these findings were supported by Albu *et al.* (1997) in a study of a slightly larger sample of obese non-diabetic premenopausal African-American and Caucasian women. After adjustment for WHR and fat mass, the African-American women had less VAT and a lower amount of VAT relative to SAT (VAT/SAT ratio). The lower levels of VAT reported in African women compared to Caucasian women were supported by similar findings from studies done in black South African women (Sumner *et al.*, 2011; Micklesfield *et al.*, 2010; Jennings *et al.*, 2009). Even amongst the black ethnicities differences were reported with African immigrants in the USA having higher VAT levels and a higher VAT/SAT ratio compared to African-Americans when adjusted for BMI (Ukegbu *et al.*, 2011). Literature regarding this aspect in black South Africans is much scarcer.

In the present study the % body fat as measured with both air-displacement plethysmography (ADP) using a BodPod[®] and bioelectrical impedance analysis (BIA) differed significantly between the genders. Compared to a suggested healthy normal range of 30.0 – 32.0% for women (Lee & Nieman, 2013), the women presented with a mean body fat % of 46.2% using the ADP and 34.9% using the BIA. The values for men were 19.4% and 27.9% respectively compared to a healthy normal cut-off point of 25.0% (Lee & Nieman, 2013). Although “normal cut-off points” for % body fat for healthy individuals are proposed, there are indications that different criteria for determining obesity should be applied to different ethnic groups (Katzmarzyk *et al.*, 2011). The Tanita body composition analyser used in this study is a simplified version of the BIA technique that uses leg-to-leg BIA and therefore actually only measures body fat in the periphery or legs and not VAT. Bioelectrical impedance is an example of a doubly indirect body composition method that relies on statistical associations between a measure of body composition and easily measurable body variables, resulting in a prediction of body fat. This method is associated with substantial bias at both individual and population level (Dewit *et al.*, 2000). Compared to the Tanita body composition analyser, measurement of body fat with ADP in this study accomplished measurement of total body fat which of course includes VAT. As an example of an indirect body composition method, ADP densitometry also relies on assumptions that might not be true, but the maximum bias in measured body fat is only approximately 3.0% (Dewit *et al.*, 2000). In this study BIA and ADP gave different % body fat values. This might be explained by the fact that body fat as measured with BIA is considered to be more inaccurate (Dewit *et al.*, 2000) than measurement of body fat with ADP (WHO expert consultation, 2004).

5.2.2 Fibrinolytic profile of the PURE 2010 study population

Most of the literature regarding PAI-1_{act} reports studies investigating Caucasians with only a few studies performed in African Americans. Until more recently, detailed data on PAI-1_{act} in black South Africans on an epidemiological level have been lacking. Evidence on ethnic differences in PAI-1_{act} levels has been reported, with both African-Americans (Festa *et al.*, 2003; Folsom *et al.*, 2001) and black South Africans (Nienaber *et al.*, 2008; Greyling *et al.*, 2007; Pieters *et al.*, 2006; Jerling *et*

et al., 1994) having lower levels compared to their Caucasian counterparts. The PURE 2005 and 2010 data are among the first reporting PAI-1_{act} levels representative of a bigger black South African population undergoing nutrition transition. In this study it was shown that the total PURE 2010 study population presented with PAI-1_{act} levels below the normal reference for healthy Caucasians as given by the manufacturers of the test procedure. It is noteworthy to mention again that these reference ranges were used in the PURE study since a healthy range for Africans has not yet been determined. Also, healthy reference ranges differ between kits due to a lack of standardisation between different companies. Several papers reported PAI-1_{act} levels for the total PURE study population in 2005 which also fell below the normal reference range (De Lange *et al.*, 2013; De Lange *et al.*, 2012; Pieters *et al.*, 2011; Pieters *et al.*, 2010).

In this study, the women had significantly higher PAI-1_{act} values compared to the men. Similar results were reported for the total PURE study population in 2005 (Pieters *et al.*, 2010). In another study, Nienaber *et al.* (2008) observed higher PAI-1_{act} in black South African girls compared to the boys. Gender differences in PAI-1_{act} levels are also reported in other studies. Compared to Caucasian women, however, several studies found Caucasian men to have higher PAI-1 levels (Van Harmelen *et al.*, 2000; Chadarevian *et al.*, 1999; Krishnamurti *et al.*, 1988; Lacroix *et al.*, 1996). In contrast to this, Wiman *et al.* (2000) and Stegnar and Pentek (1993) did not find any differences in PAI-1 levels between Caucasian men and women. De Lange *et al.* (2012) investigated the associations of CVD risk factors with PAI-1_{act} in the PURE 2005 study population and it was reported that gender explained 5% of the PAI-1_{act} variance in this population. It should be kept in mind that women had significantly higher BMI, WC and triglyceride levels than men which may influence PAI-1_{act} levels as PAI-1 levels are associated with body fat (Bastelica *et al.*, 2002; Alessi *et al.*, 2000), but even after adjustment for these, the gender differences remained. From the literature it seems as if PAI-1 is specifically related to central obesity rather than total obesity as visceral fat was found to actively produce PAI-1, more so than subcutaneous fat (Bastelica *et al.*, 2002; Alessi *et al.*, 2000; Alessi *et al.*, 1997). Waist circumference and triglyceride level have also been shown to be directly related to PAI-1_{act} (Krebs *et al.*, 2003; Bastelica *et al.*, 2002; Silveira, 2001; Alessi *et al.*, 1997; Asplund-Carlson *et al.*, 1993). Due to the known relationship between

PAI-1_{act} levels and WC and triglycerides, and the gender difference in WC and triglycerides, PAI-1_{act} levels were also adjusted for WC and triglycerides in this study. However, after the adjustment, the significant gender difference again remained. This indicates that there may be other factors contributing to the difference seen in PAI-1_{act} levels between men and women.

Until very recently no information was available regarding CLTs in black South Africans compared to relatively more literature available on Caucasians. De Lange *et al.* (2012) reported a mean CLT of 57.3 min for the total PURE study population in 2005 with women presenting with significantly longer CLTs compared to the men. Similar results were found for the 2010 data collection period. The study population had a mean CLT of 62.2 min and again, the women presented with significantly longer lysis times than the men. This gender difference is in contrast to two studies in Caucasians that found slightly longer CLTs in the men than the women (Meltzer *et al.*, 2008; Lisman *et al.*, 2005). In a study by Guimarães *et al.* (2009) employing a smaller sample size, gender was not associated with CLT. This difference in results may be related to differences in the fat distribution of the populations studied. As mentioned above, the women in the PURE study were significantly more obese than the men, while in Caucasians, obesity is also a major health concern in men. One possible reason for longer CLTs and thus reduced rates of fibrinolysis in women may be to protect them against excessive bleeding during menstruation (Davies & Kadir, 2012).

As for PAI-1, CLT has been linked to body fat. A strong relationship between CLT and BMI has been demonstrated in several studies (Guimarães *et al.*, 2009; Colucci *et al.*, 2008; Meltzer *et al.*, 2009; Meltzer *et al.*, 2008). From the available evidence it is however, not clear whether CLT is related to overall obesity or to central obesity as is the case for PAI-1_{act}. Using the PURE 2005 data, De Lange *et al.* (2012) demonstrated that CLT was more strongly associated with BMI than WC while the opposite was true for PAI-1_{act} and that after adjustment for PAI-1_{act}, CLT remained significantly longer in obese individuals. This indicates that there are other factors or mechanisms that may contribute to the association between CLT and BMI, independent of PAI-1_{act} levels. Also, after adjustment for PAI-1_{act}, BMI and triglycerides (which differed between genders), CLT remained longer in women than

men in the PURE 2010 dataset. PAI-1 levels are considered a strong predictor of CLT as it is a major inhibitor of clot lysis (Diebold *et al.*, 2008; Rau *et al.*, 2007; Lee & Huang, 2005) and therefore plays a prominent role in determining lysis rates. Several studies have indicated PAI-1 to contribute significantly to the variance in CLT. PAI-1 antigen explained 24% of the variance in CLT in a study by Meltzer *et al.* (2010b) and in agreement with this, De Lange *et al.* (2012) found that PAI-1_{act} contributed 27% to the variance in CLT in the 2005 PURE study population. The next section will now compare the relationship of PAI-1_{act} with markers of body composition to the relationship of CLT with body composition to determine whether the observed associations between CLT and body composition were independent (at least in part) of PAI-1_{act} or whether they merely reflect the association of PAI-1_{act} with body composition.

5.3 CORRELATIONS OF MARKERS OF FIBRINOLYSIS WITH DIFFERENT BODY COMPOSITION AND PHYSICAL ACTIVITY VARIABLES

5.3.1 Correlations of PAI-1_{act} with different body composition and physical activity variables

PAI-1_{act} correlated significantly with BMI, WC, waist: height ratio and the triceps skinfold in the women and the associations remained significant even after adjustment for age, alcohol consumption, HIV status and smoking. These are factors known to affect both PAI-1_{act} and anthropometric variables. Significant correlations for the same variables seen in the women were also present for the men, except that the associations were stronger for the men as indicated when correlation coefficients between genders were compared. In addition, PAI-1_{act} also correlated significantly with WHR, % body fat as measured with ADP and the other skinfolds in the men. For the men, all associations also remained significant after adjustments were made. PAI-1_{act} did not correlate significantly with physical activity in either the men or the women. Therefore in summary it can be seen that PAI-1_{act} correlated not only stronger, but also with more anthropometric variables in the men than in the women.

The weaker correlations seen in the women may be explained by the fact that women and especially obese women have proportionally more SAT and less VAT than men (Geer & Shen, 2009). Compared to SAT, VAT has been demonstrated to produce more PAI-1 *in vitro* (Bastelica *et al.*, 2002; Cigolini *et al.*, 1999; Alessi *et al.*, 1997) and plasma PAI-1 levels have been observed to correlate independently with VAT, especially in obese individuals (Ferguson *et al.*, 1998; Cigolini *et al.*, 1996; Shimomura *et al.*, 1996; Landin *et al.*, 1990; Vague *et al.*, 1989). PAI-1 is released by the larger amounts of stromal cells found in VAT compared to fewer stromal cells found in SAT (Bastelica *et al.*, 2002). Several researchers have, however demonstrated that PAI-1 expression from both VAT and abdominal SAT had a positive relationship with plasma PAI-1 and BMI (Alessi *et al.*, 2000; Eriksson *et al.*, 1999; Morange *et al.*, 1989). This suggested that both VAT and SAT components could influence plasma PAI-1 levels (Mavri *et al.*, 2001). However, abdominal SAT contains more stromal cells compared to femoral SAT (Rink *et al.*, 1996; Mauriège *et al.*, 1995), suggesting that abdominal SAT could have a stronger influence on plasma PAI-1 values compared to femoral SAT. This was confirmed by Mavri *et al.* (2001) in a study that demonstrated that PAI-1 expression in abdominal, but not in femoral SAT was associated with increased plasma PAI-1 levels in obese participants. As discussed in Section 5.2.1 obese women present with proportionally more SAT in the femoral and abdominal regions. Therefore, the higher PAI-1_{act} levels seen in the women compared to the men in this study may in part be explained by the proportionally higher abdominal SAT component, specifically in the women with a higher WC and BMI. However, despite higher PAI-1_{act} levels seen in the women than the men, proportionally lower VAT in women may explain the overall lower correlations with anthropometric variables in women compared to the men.

PAI-1_{act} was not, however, consistently associated with % body fat when comparing two methods of % body fat determination – ADP and BIA. The correlation of PAI-1_{act} with % body fat as measured with ADP was stronger than the correlation with % body fat as measured with BIA. As discussed in Section 5.2.1, measurement of % body fat in this study with BIA accomplished only measurement of body fat in the periphery or legs and not VAT but measurement of body fat with ADP provided a measurement of total body fat that included VAT. Since the association of PAI-1 with VAT is stronger than with SAT, and because total body fat, including VAT, was

not included in the measurement with BIA, this may explain the weaker correlation between PAI-1_{act} and % body fat as measured with BIA.

5.3.2 Correlations of CLT with different body composition and physical activity variables

In contrast to PAI-1_{act}, CLT correlated significantly with all the body composition variables in both men and women, except for % body fat as measured with BIA in the men. Since the association of CLT is stronger with overall obesity as indicated by BMI and because the ADP method measures total body fat, this may explain the stronger correlation observed between CLT and % body fat as measured with ADP in both genders. The significance of the associations remained after adjustment for possible confounders. The magnitude of the correlations of CLT with the body composition variables was similar between men and women. CLT did not correlate significantly with physical activity in either the men or the women.

Compared to PAI-1_{act}, CLT correlated significantly in the women with more body composition variables, and correlations of CLT with BMI, WC, waist: height ratio and triceps skinfold were significantly stronger. In the men, CLT and PAI-1_{act} correlated significantly with the same body composition variables and correlations were of the same magnitude, except for BMI which correlated significantly stronger with CLT.

These findings support the observations from the PURE 2005 data that PAI-1_{act} shows a stronger association with markers of central obesity (more specifically VAT) (Pieters *et al.*, 2010) and that CLT has a stronger association with total body fat (indicated by BMI) (De Lange *et al.*, 2012). What is of further interest is that in men the correlation between PAI-1_{act} and body composition is similar to the correlations observed between CLT and body composition (except for BMI) while in women stronger and more correlations were observed for CLT compared to PAI-1_{act}. The next section investigating the PAI-1_{act} and CLT across body composition categories may shed some light on this phenomenon.

5.4 DIFFERENCES AND COMPARISONS IN MARKERS OF FIBRINOLYSIS BETWEEN TERTILES AND CATEGORIES OF BODY COMPOSITION VARIABLES IN MEN AND WOMEN

For BMI, WC and waist: height ratio, mean PAI-1_{act} levels showed similar pictures for the men and the women with PAI-1_{act} increasing across categories. For % body fat as measured with ADP, mean PAI-1_{act} levels increased in both men and women from tertile 1 to tertile 2, and it continued to increase in tertile 3 in the men, but decreased in tertile 3 in the women. No differences across % body fat categories were observed when using BIA. Mean PAI-1_{act} levels increased significantly over the tertiles of all the skinfolds in the men, while they did not do so in women.

Mean CLT gave a similar picture in both the men and the women, increasing significantly over categories of BMI, WC (regardless of the cut-offs used) and the waist: height ratio. For % body fat as measured with ADP in the men, mean CLT decreased in tertile 2 but increased again in tertile 3 while no differences were observed in women across the categories. When measuring % body fat using BIA, mean CLT increased significantly across the tertiles in women, but only tended to increase across tertiles in the men. As discussed in Section 5.3.1, measurement of body fat with ADP in this study accomplished measurement of total body fat that includes the VAT component, whereas measurement of body fat with the leg-to-leg BIA technique only measured body fat in the lower body periphery and not the VAT component. The preferential gynoid fat distribution of women may therefore explain the increase in CLT across BIA fat % categories, while the proportionally lesser fat deposition in the periphery in men may explain the weaker association.

Mean CLT increased significantly over tertiles for all the skinfolds in both the men and women. Since PAI-1_{act} is considered to be a significant predictor of CLT, and is also strongly related to body composition, the differences in mean CLT over tertiles and categories were adjusted for PAI-1_{act}, to determine whether the associations observed with CLT are merely a reflection of the associations observed with PAI-1_{act} or whether an independent association exists. After adjustment, significance however remained. It can therefore be concluded that the significant associations of

CLT with body composition variables observed in this study are, at least in part, independent of PAI-1_{act}.

In general it seems that in men, there is a better agreement between the relationship of PAI-1_{act} with body composition and the relationship of CLT with body composition than there is in women. For example it is clear that CLT increases across skinfold categories in women, while PAI-1_{act} does not. CLT furthermore continues to increase across BMI categories while PAI-1_{act} levels seem to reach a plateau at the higher categories. In men, however, PAI-1_{act} levels increased linearly across all BMI categories. This gender difference may be explained by the difference in adipose tissue distribution between men and women as discussed in section 5.2.1. Women at higher BMI categories have proportionally more SAT in the gluteal-femoral region as they have a preferential gynoid fat deposition profile. Therefore peripheral SAT likely increases more than VAT in women which could explain why PAI-1_{act} levels plateau at the higher BMI categories – i.e. no significant increase in VAT. Compared to the women, however, men present with preferentially increased VAT deposition in the abdominal area at higher BMI categories and that may explain the linear increase in PAI-1_{act} levels, since PAI-1_{act} is expressed in higher quantities by VAT. This hypothesis also fits with our observation that CLT is more strongly related to total body fat (both VAT and SAT) while PAI-1 correlates better with central obesity (VAT and to a lesser extent abdominal SAT). Thus regardless of the type of fat deposition, CLT increases as BMI increases.

The gender differences observed for PAI-1 may, however, also be related to difference in triglyceride levels. Pieters *et al.* (2010) found that after WC, triglycerides was the main independent contributor to the variance in PAI-1_{act} levels in the PURE study population in 2005, with triglycerides explaining 5% of the variance. It was also reported that PAI-1_{act} levels were significantly higher in the participants with increased triglyceride levels than those with normal serum levels (Pieters *et al.*, 2010). In the current study the women had significantly higher triglyceride levels compared to men. Therefore high serum triglyceride levels in the women may also contribute to PAI-1_{act} levels remaining higher in the women after adjustment for WC.

An increased concentration of plasma triglycerides may contribute to increased production of PAI-1 (Krebs *et al.*, 2003), possibly through a VLDL response element identified in the promoter area of the PAI-1 gene (Eriksson *et al.*, 1998), which can induce PAI-1 transcription in endothelial cells (Eriksson *et al.*, 1998). After adjustment for triglycerides, PAI-1_{act}, however, remained statistically significantly higher in the women compared to the men. Similarly CLT was also adjusted for triglycerides and again the significance remained with CLT being significantly higher in the women than in the men. Since it is clear that CLT is not solely associated with obesity through the PAI-1 mechanistic pathway, it is necessary to investigate other possible factors that may explain the association between CLT and body fat. The following paragraphs will focus on other factors that may also explain the association between CLT and obesity.

The role of PAI-1 has been the focus of most of the research on fibrinolysis (Mertens & Van Gaal, 2002) but several other proteins may affect CLT. In 2001, Lisman and co-workers published results in which CLT was found to have a direct relationship with not only PAI-1, but also with thrombin activatable fibrinolysis inhibitor (TAFI) and α -2-antiplasmin and an indirect relationship with plasminogen. In the study by Meltzer *et al.* (2010b), 77% of the variation in CLT could be explained by this combination of fibrinolytic factors, with PAI-1 as the major determinant, followed by TAFI, then plasminogen, α -2-antiplasmin and prothrombin. TAFI is activated by thrombin to TAFI_a, a basic carboxypeptidase, which acts as an inhibitor of fibrinolysis. TAFI_a prevents binding of t-PA and plasminogen to the fibrin clot through the cleavage of lysine residues, and therefore also prevents the activation of plasminogen to plasmin (Colucci & Semeraro, 2012; Bajzar, 2000). Plasma TAFI levels have been shown to have a significant positive correlation with CLT in healthy individuals (Mosnier *et al.*, 1998; Meltzer *et al.*, 2010b). In a case-control study by Meltzer *et al.* (2009) CLT was shown to increase with increasing BMI. In addition to increasing PAI-1 levels, TAFI was offered as another possible explanation for this observed association. The researchers did not speculate regarding a possible mechanism as to how TAFI may contribute to the association between CLT and BMI (Meltzer *et al.*, 2009), except to mention that TAFI levels have also been positively associated with lipid levels and BMI (Aubert *et al.*, 2003; Silveira *et al.*, 2000). Two

other studies have also reported increased TAFI levels in obese adults (Faber *et al.*, 2009; Guven *et al.*, 2006). TAFI was unfortunately not measured in this study.

During fibrinolysis both plasminogen and t-PA bind lysine residues on the surface of the fibrin clot (Gale, 2010; Rau *et al.*, 2007). Through cleavage of plasminogen at these binding sites by t-PA, plasminogen is activated to plasmin (Gale 2010; Rau *et al.*, 2007), resulting in the degradation of the clot into small fibrin fragments (Mosnier & Bouma, 2006). A longer CLT was found to be associated with a decreasing level of plasminogen and consequently plasmin (Meltzer *et al.*, 2010b). Alpha-2-antiplasmin is an inhibitor of the fibrinolytic pathway and functions through inhibition of plasmin (Gale, 2010; Rijken & Lijnen, 2009; Rau *et al.*, 2007). Increased α -2-antiplasmin levels have been associated with longer CLTs (Meltzer *et al.*, 2010a). The following discussion will focus on the available evidence which can provide a possible link between plasminogen and α -2-antiplasmin and obesity, contributing to the explanation of the observed association between CLT and obesity.

Meltzer *et al.* (2010a) investigated the associations between plasma levels of fibrinolytic proteins, CVD risk factors and the risk of myocardial infarction in men. In this study α -2-antiplasmin was positively and independently associated with TC and BMI. Another study conducted by Michalska *et al.* (2013) also reported a relationship between α -2-antiplasmin and obesity. A higher activity of α -2-antiplasmin was observed in patients with morbid obesity (BMI > 40 kg/m²). In a study by Meltzer *et al.* (2010a), plasminogen was shown to be positively and independently associated with smoking, CRP, triglycerides, TC levels and BMI.

Plasminogen is furthermore one of several acute-phase proteins that increase during inflammatory processes (Haverkate *et al.*, 1995; Jenkins *et al.*, 1997). Obesity is one of several metabolic syndrome-associated disorders where several factors elicit and contribute to an inflammatory response. Adipocytes release mediators that induce a chronic inflammatory state with subsequent alterations in blood coagulation and fibrinolysis (Samad & Ruf, 2013; Stoppa-Vaucher *et al.*, 2012). Since both plasminogen and α -2-antiplasmin affect CLT, the limited evidence that plasminogen and α -2-antiplasmin levels are affected by obesity (and inflammation) may provide another possible pathway through which CLT is associated with BMI.

Besides the abovementioned fibrinolytic proteins, prothrombin is a coagulation protein shown to be an important determinant of CLT (Lisman *et al.*, 2001). Prothrombin functions in the initiation phase of blood coagulation (Monroe & Hoffman, 2006). Activated factor X (FXa) binds activated factor V (FVa) and this converts prothrombin to thrombin. Thrombin eventually converts fibrinogen to fibrin during the propagation phase of blood coagulation (Hoffman & Monroe, 2007; Monroe & Hoffman, 2006; Cesarman-Maus & Hajjar, 2005). Increased prothrombin levels were found to be associated with longer CLTs (Meltzer *et al.*, 2010a). The researchers who reported these results (Meltzer *et al.*, 2010a) suggested that the relationship between prothrombin and CLT can probably be explained by an increased activation of TAFI to TAFIa by thrombin. There is also some evidence in the literature of a link between thrombin and its precursor prothrombin and obesity. Significantly shorter blood coagulation in terms of prothrombin time and thrombin time was reported from a study performed in visceral obese animal models. The researchers from this study concluded that these results suggest that visceral obesity is related to accelerated blood coagulation (Kaji *et al.*, 2013). In 2012, a study by Stoppa-Vaucher and co-workers reported a shortened prothrombin time and an increased endogenous thrombin generation potential (ETP) in obese children of European descent. Other studies also demonstrated that ETP was significantly increased in young overweight and obese subjects (Fritsch *et al.*, 2010; Cimenti *et al.*, 2006) and in adults with obesity associated with stroke (Sarıkaya *et al.*, 2011) and myocardial infarction (Smid *et al.*, 2011). Also in adults, BMI was found to be positively associated with ETP in women with venous thromboembolism (Sonnevli *et al.*, 2013). In a study by Prüller *et al.* (2011) ETP was significantly higher in the overweight and obese but otherwise healthy subjects compared to the controls. Brummel-Ziedins *et al.* (2005) studied thrombin generation in healthy individuals and all four thrombin parameters that were studied were affected by BMI. From a study in 2007, Peverill *et al.* reported that the markers of thrombin generation, prothrombin fragment 1 and 2 (F1 and F2) correlated positively with BMI, WC and WHR in healthy postmenopausal women. An increase in F1 and F2 with higher BMI was also reported by Rugman *et al.* (1994).

The structure of the fibrin clot that forms upon activation of the coagulation system is another factor thought to influence CLT (Weisel 2007, Undas *et al.*, 2006, Chernysh & Weisel 2008, Scott *et al.*, 2004). A longer CLT time is seen with dense clots composed of thinner fibres compared to less dense clots composed of thicker fibres (Collet *et al.*, 2003; Collet *et al.*, 2000). Clot density and CLT are related to the presence of the metabolic syndrome and were demonstrated to increase with an increase in the number of metabolic syndrome components (Carter *et al.*, 2007). From a study done by Malan (1999) it appeared that obese African women had fibrin network structures that may be more thrombogenic compared to the normal weight women. This type of clot structure is associated with a longer CLT. It might therefore be hypothesized that since the clot structure of obese individuals seems to be denser and thus more atherogenic, it may also explain longer CLTs seen in the participants with overweight and obese BMIs in the present study. A study done by Brzezinska-Kolarz *et al.* (2013) furthermore demonstrated that the efficiency of fibrin clot lysis was increased with weight loss in obese people. Significant reductions in both PAI-1, associated with visceral adipose tissue, and t-PA were seen in the study. Since a decrease in both PAI-1 and t-PA can modulate fibrin properties (Undas, 2011; Hoffman, 2008; Scott *et al.*, 2004), these two factors were suggested as possible mechanisms through which they might accelerate the lysis of clots (Brzezinska-Kolarz *et al.*, 2013).

Compared to the men, PAI-1_{act} showed very little association with most of the skinfolds in the women except with the triceps skinfold (see Table 4.2), while in men there was a strong positive association. When looking at the tertile cut-off values for men and women, it is clear that the skinfolds of the men are much smaller than those of the women and that the skinfolds of the women start at much higher values compared to the men. Therefore, it is possible that the association between skinfolds and PAI-1_{act} may have a similar relationship to that of PAI-1_{act} with BMI, in that the increase is not linear, but plateaus at higher skinfold values. It can therefore be speculated that the apparent lack of association between PAI-1_{act} and skinfold thickness in women may be due to the fact that skinfold values fall within the plateau-level of the curve while in men, due to the lower absolute skinfold thickness, these values still fall within the linear part of the curve and PAI-1_{act} levels may therefore still be sensitive to the change or increase in skinfold thickness and the

amount of fat tissue associated with it. Figure 4.2 clearly illustrates this gender difference in the association of PAI-1_{act} with the triceps skinfold.

The difference in sequence and pattern of body fat accumulation between men and women may, however, also contribute to these observed gender differences. Skinfolds represent SAT and men tend to accumulate VAT first before an increase in SAT is seen. This may explain why PAI-1_{act} levels continue to rise across skinfold quintiles for the men as more VAT would have been deposited before an increase in SAT is observed. Since women tend to store more peripheral SAT, this adipose compartment will increase in accordance with an increase in skinfold. Proportionally their VAT compartment will be smaller and this may explain why PAI-1_{act} levels plateau and do not continue to increase across the higher skinfold quintiles. In contrast to this, a longer lysis time is observed for both men and women as skinfold values continue to increase. This again provides evidence that CLT is at least in part independent of PAI-1_{act} associated with body fat and that it is associated with total body fat rather than a specific type of fat.

5.5 LIMITATIONS

In this study several factors that might have added to the understanding of the difference in association between PAI-1_{act} and CLT with body composition markers were not determined, i.e. other haemostatic variables, TAFI, prothrombin, α -2-antiplasmin and plasminogen. Since it was not possible to differentiate clearly between abdominal SAT and VAT (apart from using differences in skinfold measurements at abdominal and peripheral positions between men and women), a limitation was placed on the attempt to describe the association between PAI-1_{act} and abdominal obesity in more detail, especially the observed difference in this association between men and women.

5.6 CONCLUSION

It can be concluded that this mini-dissertation supports observations made during baseline data collection in 2005 in the PURE study that PAI-1_{act} shows a stronger association with central obesity while CLT has a stronger association with total body

fat. We additionally indicated that the association of PAI-1_{act} and CLT with body composition was similar in men, but that in women the two fibrinolytic variables showed different associations with body composition markers. Differences in adipose tissue distribution and sequence of accumulation between men and women may explain the gender differences that were observed in these associations. This is the first study to explore the reported relationship between components of the fibrinolytic system and body composition in more depth in order to identify mechanistic detail. Additionally, until more recently, detailed data on PAI-1_{act} and CLT in black South Africans on an epidemiological level have been lacking. Findings from this study contribute to a better understanding of the relationship between PAI-1_{act} and CLT as markers of plasma fibrinolytic potential and body composition in this population. It can also be concluded that the significant associations observed between CLT and body composition variables are, at least in part, independent of PAI-1_{act}. We provide several other postulated mechanisms that may explain the link between CLT and body composition. Our results indicate that both total and central obesity may increase CVD risk partly through ineffective clot lysis but that the mechanism of action may differ between the two conditions.

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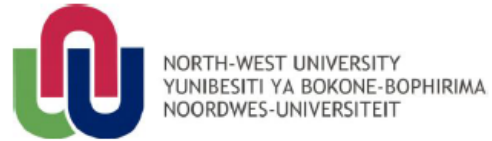
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ADDENDA

ADDENDUM A
ETHICAL APPROVAL PURE 2010



Private Bag X6001, Potchefstroom
South Africa 2520

Tel: (018) 299-4900
Faks: (018) 299-4910
Web: <http://www.nwu.ac.za>

ETHICS APPROVAL OF PROJECT

Ethics Committee
Tel +27 18 299 4850
Fax +27 18 293 5329
Email Ethics@nwu.ac.za

2010-02-23

This is to certify that the next project was approved by the NWU Ethics Committee:

Project title : <i>PURE study (Prospective Urban and Rural Epidemiology study)</i>	
Project leader / Student : Prof Annamarie Kruger	
Ethics number:	NWU-00016-10-A1
<small><u>Status:</u> S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation</small>	
Expiry date: 20/01/2015	

The Ethics Committee would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the Ethics Committee for any further enquiries or requests for assistance.

The formal Ethics approval certificate will be sent to you as soon as possible.

Yours sincerely



Me. Marietjie Halgryn
NWU Ethics Secretariate

ADDENDUM B
INFORMED CONSENT FORM PURE 2010



PURE-SA Project (Prospective Urban and Rural Epidemiology)
INFORMED CONSENT FORM (including the PRIMER-study)

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

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

I hereby also declare that I am aware that:

1. This blood sample will be used for the purpose of
 - a. Isolating DNA to look at genetic factors that are currently associated with Type 2 Diabetes (i.e. the Calpain10, Adiponectin, Leptin and Leptin Receptor genes), or genetic factors that may be associated with Non Communicable diseases in the future. We give the assurance that all genetic tests and experiments will only focus on genotypes suspected to contribute to an increased risk of non communicable diseases of lifestyle.
 - b. DNA damage due to environmental factors such as cooking methods and smoke will be tested for.
 - c. Testing for liver function by determining liver enzymes such as AST, GGT,
 - d. Analyses of other than genetic parameters for Diabetes Mellitus such as HbA_{1c}, Blood glucose and Insulin
 - e. Analyses of the clotting profile and hypertension markers
 - f. Analyses of bone health, iron and nutrition status

And may be stored until such time as the above measurements/analyses will be done.
2. Body measurements such as height, weight, skin fold thicknesses, arm and leg circumferences will be taken
3. Vascular sonar will be done
4. Blood pressure to be taken
5. Pulse wave velocity measurements will be made
6. A Spirometer test to be performed to determine lung function
7. Bone density will be determined with an osteometer to detect possible osteoporosis

.....
(Signature of the subject)

Signed at ... Potchefstroom / Ganyesa ... (delete not applicable option) on/...../ 2010

Witnesses

1. 2.

Signed at ... Potchefstroom / Ganyesa ... (delete not applicable option) on/...../ 2010

PART 1

- 1. Research Unit and Faculty:**
Africa Unit for transdisciplinary Health research (AUTHeR); Faculty of Health Sciences, North-West University
- 2. Title of project/trial:**
PURE: Prospective Urban and Rural Epidemiological study
- 3. Full names, surname and qualifications of project leader:**
Prof. Annamarie Kruger, Ph.D. (Nutrition)
- 4. Rank/position of project leader:**
Research Director and project manager
- 5. Aim of this project**
PURE's aim is that understanding the different lifestyle and health transitions of individuals in response to societal changes will elucidate societal and individual adaptive strategies that could diminish the adverse health effects of industrialization and urbanization on health, while retaining its benefits.
- 6. Explanation of the nature of all procedures, including identification of new procedures:**
Each participant will have to fill in a number of questionnaires (Adult questionnaire, Physical activity questionnaire, Food frequency questionnaire, Health questionnaire, psychology questionnaire) with the help of field workers. A blood sample will be taken. Physical measures will be performed, including anthropometric measures (such as weight, height, and waist circumference), blood pressure, lung capacity and lung volume and bone density will be performed.
- 6. Description of the nature of discomfort or hazards of probable permanent consequences for the subjects which may be associated with the project: (Including possible side-effects of and interactions between drugs or radio-active isotopes which may be used.)**
It will take each participant quite a while (about 5 [six] hours) to complete all the tests and questionnaires. Discomfort may be experienced with the taking of blood samples. No measures will have permanent damage or consequences for the participants.
- 8. Precautions taken to protect the subjects:**
The research nurse will be present at all times, and will be responsible for the blood sampling. She is very experienced and has performed these procedures numerous times in previous studies.
- 9. Description of the benefits which may be expected from this project:**
When measures with immediate results are taken, such as blood glucose, haemoglobin, HIV status and blood pressure, the information will be communicated to the individual to seek professional help during an individual feedback session. Since this study is a longitudinal study, subjects that are high at risk will be identified over time and personal feedback and counselling will be given.
On the research site each participant will receive a cooked meal, a fruit and fruit juice. Also an amount of R50 will be paid to each participant to cover transport costs and enable each person to go for follow-up at a clinic of choice.
- 10. Alternative procedures which may be beneficial to the subjects:**
There will be tested for HIV/AIDS, therefore pre-test counselling will be given. If the subject wants to know his/her status and he/her tests positive, post counselling will also be given.

PART 2

To the subject signing the consent:

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

- 1. Participation in this project is voluntary.**
- 2. It is possible that you personally will not derive any benefit from participation in this project, although the health knowledge obtained from the results may be beneficial to you and other people.**
- 3. You will be free to withdraw from the project at any stage without having to explain the reasons for your withdrawal. However, we would like to request that you would rather not withdraw without a thorough consideration of your decision, since it may have an effect on the statistical reliability of the results of the project.**
- 4. The nature of the project, possible risk factors, factors which may cause discomfort, the expected benefits to the subjects and the known and the most probable permanent consequences which may follow from your participation in this project, are discussed in Part 1 of this document.**
- 5. We encourage you to ask questions at any stage about the project and procedures to the project leader or the personnel, who will readily give more information. They will discuss all procedures with you.**
- 6. The University staff will use standardised procedures and take all possible precaution to protect the subject from risks.**
- 7. All information will be kept CONFIDENTIAL and no personal information will be published without my consent.**

Prof ANNAMARIE KRUGER

Contact details: 082 771 5778 / 018 299 2095(Office)

ADDENDUM C
ADULT QUESTIONNAIRE

PURE/South Africa

We are very grateful to you for your participation in this study. All information given by you will be held in strict confidence, and will be used for the purpose of this study only after removing any personal identifying information.

Adult Questionnaire

INSTRUCTIONS

Please answer EACH question by marking an X in ONE BOX on each line:
(unless otherwise instructed)



OR

By writing number(s) in the spaces provided:



OR

By specifying the answer on the line(s) provided

April 28, 2005

Adult Questionnaire

Subject Initials- F= first letter of first name
M= first letter of middle name
L= first letter of last name

3. National I.D#

If not applicable please mark the N/A box

Ethnicity Codes

- 01 - South Asian (India, Sri Lanka, Pakistan, Bangladesh)
- 02 - Chinese (China, Hong Kong, Taiwan)
- 03 - Japanese
- 04 - Malays
- 05 - Other Asian (Korea, Malaysia, Papua New Guinea, Thailand, Philippines, Indonesia, Nepal, Vietnam, Cambodia, Laos, Myanmar/Burma, Bhutan, Singapore)
- 06 - Persian
- 07 - Arab
- 08 - Black African
- 09 - Coloured African (Subsaharan African only)
- 10 - European
- 11 - Native North/South American or Australian Aborigine
- 12 - Latin American (Latino)
- 13 - Bantu/Semi Bantu
- 14 - Hemitic/Semi Hemitic
- 15 - Nilotic/Hausa
- 16 - Pygmie
- 17 - Swahili
- 18 - Other (any other ethnoracial group not listed above)

Subject ID

Centre # Community# Household # Subject #

Subject Initials
 F M L

Today's date:
 year month day

1. Name: _____
 Given name Surname

2. Not applicable in South Africa

3. National identity # or equivalent: _____ N/A

4. DOB: OR Age yrs

5. Sex: Female Male

6. Marital status: (check one only)

Never married Currently married Common law/Living with partner
 Widowed Separated Divorced

7. Ethnicity: → (Please refer to facing page for codes)

8. Caste/Tribe: _____

9. What level of formal education have you **completed**? (check highest level only):

- None
- Primary
- Secondary/highschool/higher secondary
- Trade School
- College/University
- Unknown

Adult Questionnaire

11. Occupation

Group 1: Legislators, senior officials and managers

- Legislators and senior officials
- Corporate managers
- General managers
- Businessman

Group 2: Professionals

- Physical, mathematical and engineering science professionals
- Life science and health professionals
- Teaching professionals
- Other professionals

Group 3: Technicians and associate professionals

- Physical, mathematical and engineering-science associate professionals/technicians
- Life science and health associate professionals/technicians
- Teaching associate professionals/technicians
- Other associate professionals/technicians

Group 4: Clerks

- Clerks
- Customer service clerks

Group 5: Service workers and shop and market sales workers

- Personal and protective services workers
- Models, salespersons and demonstrators

Group 6: Skilled agricultural and fishery workers

- Market-oriented skilled agricultural and fishery workers
- Subsistence agricultural and fishery workers

Group 7: Craft and related trade workers

- Extraction and building trade workers
- Metal, machinery and related trades workers
- Precision, handicraft, printing and related trades workers
- Other craft and related trades workers

Group 8: Plant and machine operators and assemblers

- Stationary plant and related operators
- Machine operators and assemblers
- Drivers and mobile plant operators

Group 9: Elementary occupations

- Sales and services elementary occupations
- Agricultural, fishery and related labourers
- Labourers in mining, construction, manufacturing and transport

Group 10: Armed forces

- Armed forces

Group 11: Homemaker

- Housewife/Househusband

Subject ID

Centre # Community# Household # Subject #

Subject Initials
 F M L

10. Not applicable in South Africa

11a) Not applicable in South Africa

b) Please indicate which group best describes your main occupation.
 (Please refer to facing page for definitions of groups and instruction manual for detailed definitions)

Group 1 Group 2 Group 3 Group 4 Group 5
 Group 6 Group 7 Group 8 Group 9 Group 10 Group 11

c) Not applicable in South Africa

d) What is your main source of income? _____

If occupation is group 11 (homemaker) go to question 13

12. Are you currently employed?

No → (answer 12a - 12b) Yes → Go to #13

a) Are you retired/stopped work from your primary occupation due to old age? No Yes

b) Have you stopped working due to illness? No Yes

Subject ID

Centre # Community# Household # Subject #

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13. CURRENT DISABILITY:

	No	Yes
a) Do you have any problems using your fingers to grasp or handle?	<input type="checkbox"/>	<input type="checkbox"/>
b) Do you have any trouble walking about?	<input type="checkbox"/>	<input type="checkbox"/>
c) Do you have any trouble bending down and picking up an object from the floor?	<input type="checkbox"/>	<input type="checkbox"/>
d) Do you require a walking stick cane/walker to move about?	<input type="checkbox"/>	<input type="checkbox"/>
e) Do you have any trouble reading or seeing the individual grains of rice/corn on your plate? (with glasses worn)	<input type="checkbox"/>	<input type="checkbox"/>
f) Do you have trouble seeing a person from across the room? (12 feet/3.5 meters) (with glasses worn)	<input type="checkbox"/>	<input type="checkbox"/>
g) Do you have trouble speaking and being understood?	<input type="checkbox"/>	<input type="checkbox"/>
h) Do you have any trouble hearing what is said in a normal conversation?	<input type="checkbox"/>	<input type="checkbox"/>

Subject Medical History

14. Have you experienced any of the following in the last six months?

	No	Yes		No	Yes
a) Chest pain or tightness with usual activity	<input type="checkbox"/>	<input type="checkbox"/>	i) Vomiting	<input type="checkbox"/>	<input type="checkbox"/>
If Yes, → does the pain spread to the back, neck or inner border of arm	<input type="checkbox"/>	<input type="checkbox"/>	j) Loss of appetite	<input type="checkbox"/>	<input type="checkbox"/>
b) Breathlessness with usual activity	<input type="checkbox"/>	<input type="checkbox"/>	k) Painful or bleeding teeth/gums	<input type="checkbox"/>	<input type="checkbox"/>
c) Cough for at least 2 weeks	<input type="checkbox"/>	<input type="checkbox"/>	l) Jaundice	<input type="checkbox"/>	<input type="checkbox"/>
d) Any sputum while coughing	<input type="checkbox"/>	<input type="checkbox"/>	m) Burning while passing urine	<input type="checkbox"/>	<input type="checkbox"/>
e) Blood in sputum	<input type="checkbox"/>	<input type="checkbox"/>	n) Swelling of feet	<input type="checkbox"/>	<input type="checkbox"/>
f) Wheezing or whistling in the chest	<input type="checkbox"/>	<input type="checkbox"/>	o) Swelling of face	<input type="checkbox"/>	<input type="checkbox"/>
g) Early morning cough with chest tightness	<input type="checkbox"/>	<input type="checkbox"/>	p) Blood in urine	<input type="checkbox"/>	<input type="checkbox"/>
h) Loose stools/diarrhea for at least 3 days	<input type="checkbox"/>	<input type="checkbox"/>	q) Involuntary weight loss of > 3kg	<input type="checkbox"/>	<input type="checkbox"/>

15. Not applicable in South Africa

16a) Do you use glasses/spectacles/contact lenses at present? No Yes

b) Do you use a hearing aid? No Yes

Adult Questionnaire

Cancer Sites

- 1= Mouth
- 2= Esophagus
- 3= Stomach
- 4= Small intestine
- 5= Large intestine including rectum
- 6= Pancreas
- 7= Liver
- 8= Lung
- 9= Breast
- 10= Cervical/uterine/ovarian
- 11= Prostate
- 12= Head and neck
- 13= Other, specify

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17. Have you ever been diagnosed with any of the following?(check all that apply)

	No	Yes	#of yrs since diagnosis		No	Yes	#of yrs since diagnosis
a) Diabetes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	i) COPD	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
b) Hypertension/ high blood pressure	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	j) Asthma	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
c) Stroke	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	k) Tuberculosis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
d) Angina/heart attack/ Coronary artery disease	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	l) Malaria	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
e) Heart failure	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	m) Chagas	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
f) Other heart disease	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	n) HIV/AIDS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
g) Cancer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>				

Please refer to facing page for cancer sites site other, specify

18. Have you been taking any medications regularly (ie. at least once per week) in the last month? No → go to 19 Yes

a) If yes, for what conditions:

	No	Yes
Blood pressure	<input type="checkbox"/>	<input type="checkbox"/>
Cholesterol lowering drugs	<input type="checkbox"/>	<input type="checkbox"/>
Stroke	<input type="checkbox"/>	<input type="checkbox"/>
Diabetes	<input type="checkbox"/>	<input type="checkbox"/>
Asthma	<input type="checkbox"/>	<input type="checkbox"/>
Chinese medicine	<input type="checkbox"/>	<input type="checkbox"/>
Others	<input type="checkbox"/>	<input type="checkbox"/> → If Yes, specify _____
Unknown	<input type="checkbox"/>	<input type="checkbox"/>

Adult Questionnaire

18b) If name of medication is unknown, please list as unknown.

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18b) List all the medications you are currently consuming at least once a week for the last month?

- i) _____ ii) _____
iii) _____ iv) _____
v) _____ vi) _____
vii) _____ viii) _____

Men go to question #23

For Women Only (Questions 19 - 22)

19. Are you currently pregnant? No Yes → Go to #21

20. Do you still have periods? No → (answer 20a) Yes → Go to #21

a) How many years since you stopped menstruating? years

21. Have you ever used an oral/ injectable contraceptive? No Yes

22a) How many live children have you given birth to? Boys Girls

b) Did you breast feed any of your children? No Yes

Adult Questionnaire

23. Accidents and Injuries

Location of Injury

- 1= Factory/industrial place
- 2= Office
- 3= Agriculture field/farm
- 4= Home
- 5= Road
- 6= Sport/game e.g. track, court, field, etc.
- 7= Public building
- 8= Mine/quarry
- 9= Construction site e.g. building, road-works, etc.
- 10 = Other

Type of Injury

- 1= Burns
- 2= Scalds
- 3= Fractures
- 4= Muscle and ligament sprains/tears
- 5= Cuts and lacerations
- 6= Bruises and abrasions
- 7= Suffocation
- 8= Head injury (where person did not lose consciousness)
- 9= Head injury (where person lost consciousness for some time)

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23. During the past 12 months, have you had any injuries that were serious enough to limit your normal activities? (check all that apply) No → Go to #24 Yes → (answer 23a - 23s)

If yes, please provide details:

Please refer to facing page for Location and Type Codes
Absence from work or usual activities (Days)

Cause of injury	No	Yes	Location	Type	Absence from work or usual activities (Days)
a) Motor vehicle accident (as a passenger)	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
b) Motor vehicle accident (as a pedestrian)	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
c) Struck by an object	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
d) Explosion	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
e) Natural/environmental factors (gales/cyclones/lightning, etc.)	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
f) Suffocation	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
g) Poisoning	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
h) Snake/scorpion bite	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
i) Fall	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
j) Fire/flames, resultant fumes	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
k) Physical assault (gun, kidnapping, etc.)/violent crime	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
l) Domestic violence (beaten by a family member)	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
m) Drowning/submersion	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
n) Hot or corrosive liquids/floods/substances	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
o) Crush injuries (boulders, building materials, etc.)	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
p) Accident caused by machinery	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
q) Attempted suicide	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
r) Armed conflict	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
s) Other(specify) _____	<input type="checkbox"/>	<input type="checkbox"/>	→ <input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>

Adult Questionnaire

Location of Fractures

- 1= Hip/pelvis
- 2= Thigh
- 3= Leg
- 4= Forearm
- 5= Wrist
- 6= Hand/finger
- 7= Vertebrae (back)
- 8= Other

Fractures: In situations where subjects are in a cast and cannot differentiate between ligament tear or fracture, include as fracture only if doctor confirmed it as a broken bone

25c) Tobacco: Regular use is defined as consuming at least one tobacco product per day.

Duration of use:

For those that have consumed tobacco for <1 year, please enter "0"

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24. Have you ever fractured a bone? No (go to #25) Yes (if yes, answer a,b) and c)

a) Number of fractures

b) Years since last fracture (yrs)

c) Bone (s) broken in the most recent fracture(if more than 3, list most severe sites) (location) If other, specify

Please refer to facing page for fracture locations →

Tobacco →

25. Which best describes your history of tobacco use?

a) Formerly used tobacco products Currently use tobacco products Never used tobacco products → Go to #26

b) At what age did you start? yrs

c) Have you ever regularly used any of the following tobacco products? (check all that apply)

	Average amount/day	Duration (years)	When Stopped (years ago)	Past users only If less than 1 yr (months ago)
(i) Cigarettes (all kinds)	<input type="text"/> <input type="text"/> number	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(ii) Beedies	<input type="text"/> <input type="text"/> number	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(iii) Cigars	<input type="text"/> <input type="text"/> number	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(iv) Pipes	<input type="text"/> <input type="text"/> number	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(v) Sheesha/water pipe Hookah	<input type="text"/> <input type="text"/> # of times	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(vi) Chewing tobacco	<input type="text"/> <input type="text"/> # of times	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(vii) Snuff	<input type="text"/> <input type="text"/> # of times	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(x) Other <i>Specify</i> _____	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>

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<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
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Subject Initials	<input type="text"/>	<input type="text"/>	<input type="text"/>
	F	M	L

Question 26 to be answered by non-smokers and former smokers only

26. During the past 12 months, have you been regularly (at least once per week) exposed to other people's tobacco smoke?
(“Exposed” is defined as a minimum of 5 consecutive minutes, during which you inhale other people's smoke.)

No → Go to #27 Yes → Please answer questions 26a

a) Over the past 12 months, what has been your typical exposure to other peoples smoke?
(“Exposed” is defined as a minimum of 5 consecutive minutes, during which you inhale other peoples smoke)

Select **ONE** only

1-2 times/week 3-6 times/week at least once a day 2-3 times/day 4 or more times/day

27. Not applicable in South Africa

Adult Questionnaire

28c) Alcoholic Beverage: Regular use is defined as at least once a month.

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30. Are you a member of any of the following:

How often do you participate in the activities of this group?

Per Month OR Per Year

- (i) Self help group, Co-operative, Social club, Sports club, No Yes →
- (ii) Religious Group (e.g: church group, etc.) No Yes →
- (iii) Other Specify No Yes →

31. Please answer the following: (choose only one option for each)

- | | Strongly Disagree | Somewhat Disagree | Somewhat Agree | Strongly Agree |
|---|--------------------------|--------------------------|--------------------------|--------------------------|
| (i) People are generally honest and want to help others. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (ii) If I do nice things for someone, I can anticipate that they will respect me and treat me just as well as I treat them. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

32a) The television, radio, newspaper or magazine advertisements help me decide to buy the type of: (choose only one option for each) Not Applicable

- | | Strongly Disagree | Somewhat Disagree | Somewhat Agree | Strongly Agree | Not Applicable |
|------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| (i) Cooking oil | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (ii) Flour | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (iii) Rice/ Maize meal | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

b) The television, radio, newspaper or magazine advertisements influence whether I buy: (choose only one option for each)

- | | Strongly Disagree | Somewhat Disagree | Somewhat Agree | Strongly Agree | Not Applicable |
|------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| (i) Soft drinks | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (ii) Snacks | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (iii) Cigarettes | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (iv) Alcohol | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

33. In a difficult situation, whose help can you count on from?(Please see facing page for definitions)

(i) Civic organizations: specify _____
 none little moderate/average a great deal

(ii) Religious organizations: specify _____
 none little moderate/average a great deal

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34. Have you experienced any of the following events during the last 12 months?

- | | No response | No | Yes |
|---|--------------------------|--------------------------|---|
| (i) Loss of job | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (ii) Retirement | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (iii) Loss of crop/business failure | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (iv) Household break in | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (v) Marital separation/divorce | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (vi) Other major intra-family conflict | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> → Please specify _____ |
| (vii) Major personal injury or illness | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (viii) Violence | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (ix) Armed conflict/war | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (x) Death of a spouse | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (xi) Death/major illness of another close family member | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (xii) Other major stress | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> → Please specify _____ |
| (xiii) Wedding of family member | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (xiv) New job | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (xv) Birth in the family | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (xvi) Separation from family | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| (xvii) Unavailability of food/ food insecurity | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

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35. Please answer the following: (Choose only one option for each)

For the following question, stress is defined as feeling irritable or filled with anxiety, or as having sleeping difficulties as a result of conditions at work or at home.

	No response	Never Experienced Stress	Some Period of Stress	Several Periods of Stress	Permanent Stress
a) How often have you felt stress at work in the last 12 months? (Mark here if not applicable: i.e. no longer working <input type="checkbox"/>)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b) How often have you felt stress at home in the last 12 months?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

36. What level of financial stress have you felt in the last 12 months?

No response
 Little/none
 Moderate
 High/severe

37. During the past twelve months, was there ever a time when you felt sad, blue, or depressed for two weeks or more in a row?

No Yes → If yes, during those times, did you:

	No response	No	Yes
a) Lose interest in most things like hobbies, work or activities that usually give you pleasure?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b) Feel tired or low on energy?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c) Gain or lose weight?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
d) Have more trouble falling asleep than you usually do?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
e) Have more trouble concentrating than usual?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
f) Think a lot about death (either your own, someone else's, or death in general)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
g) Feel down on yourself, no good or worthless?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

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38. Please answer the following: (Choose only one option for each)

	Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree
a) I can do most of my regular shopping (food, household necessities, etc.) at stores within easy walking distance (less than 15 minutes) of my home.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b) Walking or bicycling in my neighbourhood is difficult because of the speed and/or amount of traffic.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c) My neighbourhood is generally free from pollution (litter, air pollution and noise pollution).	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
d) My neighbourhood streets are well lit at night.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
e) I can see other people when I am walking in my neighbourhood.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
f) I can speak to other people when I am walking in my neighbourhood.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
g) There is a high crime rate in my neighbourhood.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
h) There is a problem with unattended dogs in my neighbourhood.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

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38a) Please answer the following: (Please check all that apply)

i) Has your household been a victim of the following crime(s) in the last 12 months?

	No	Yes
1. Armed robbery	<input type="checkbox"/>	<input type="checkbox"/>
2. Violent attacks	<input type="checkbox"/>	<input type="checkbox"/>
3. Murder	<input type="checkbox"/>	<input type="checkbox"/>
4. Vehicle hijacking	<input type="checkbox"/>	<input type="checkbox"/>
5. House breaking	<input type="checkbox"/>	<input type="checkbox"/>
6. Theft	<input type="checkbox"/>	<input type="checkbox"/>
7. Rape	<input type="checkbox"/>	<input type="checkbox"/>
8. Women abuse eg. (beat,swear-words,sexual) please specify _____	<input type="checkbox"/>	<input type="checkbox"/>
9. Child abuse eg. (burn,swear-words,rejection) please specify _____	<input type="checkbox"/>	<input type="checkbox"/>
10. Child sexual abuse	<input type="checkbox"/>	<input type="checkbox"/>
11. Other, please specify _____	<input type="checkbox"/>	<input type="checkbox"/>

ii) Do you think that crime in your area has increased in the past 5 years? No Yes
if yes, which of the following crime(s)?

- Armed robbery
- Violent attacks
- Murder
- Vehicle hijacking
- House breaking
- Theft
- Rape
- Women abuse
- Child abuse
- Child sexual abuse
- Other, please specify _____

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38b) Questions on HIV:

i) Do you know people who have HIV/AIDS? No Yes

if yes, which of these people: (please mark all that apply)

- Your children
- Your grandchildren
- Your spouse
- Your family members
- Your friends
- People in the community

ii) What would you consider the mean age of the people who are ill/have died of HIV/AIDS?

- Younger than 10 years
- Between 11-20 years
- Between 21-30 years
- Between 31-40 years
- Between 41-50 years
- Over 50 years

iii) If someone in your household is HIV positive, who is the primary caregiver?

- Spouse
- Parents
- Family member
- Child children
- Friends
- Volunteer

38c) Do you care for any orphans in your family? No Yes

Adult Questionnaire

40b) Health History:

Cancer Sites

- 1= Mouth
- 2= Esophagus
- 3= Stomach
- 4= Small intestine
- 5= Large intestine including rectum
- 6= Pancreas
- 7= Liver
- 8= Lung
- 9= Breast
- 10= Cervical/uterine/ovarian
- 11= Prostate
- 12= Head and neck
- 13= Other, specify

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39. How long would it take you to get from your house to the nearest facility if you walked?

	Minutes	Don't know		Minutes	Don't know
i) grocery/convenience store	<input type="text"/>	<input type="checkbox"/>	iv) video store	<input type="text"/>	<input type="checkbox"/>
ii) bank	<input type="text"/>	<input type="checkbox"/>	v) non-fast food restaurant	<input type="text"/>	<input type="checkbox"/>
iii) post office	<input type="text"/>	<input type="checkbox"/>	vi) fast food restaurant	<input type="text"/>	<input type="checkbox"/>

40a) Total number of siblings

b) Health History: Complete for all parents and siblings, alive or dead

	Father			Mother			Siblings			# of siblings with the condition
	Unknown	No	Yes	Unknown	No	Yes	Unknown	No	Yes	
Diabetes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	If yes → <input type="text"/>
Coronary Heart Disease	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
High Blood Pressure	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
Stroke	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
Cancer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
	if Yes, indicate site <input type="text"/>			if Yes, indicate site <input type="text"/>			if Yes, indicate site <input type="text"/>			
	Other, Specify			Other, Specify			Other, Specify			

Please refer to facing page for cancer sites

Adult Questionnaire

If subject refuses to provide any of the measures, enter a value of "0" into each of the boxes for that question

For more detailed instructions please refer to the instruction manual

PURE

Adult Questionnaire

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Subject ID

Centre # Community# Household # Subject #

Subject
 Initials
 F M L

41. Physical Measurements

Sitting #1 mmHg
 a) Right arm blood pressure #2 mmHg
 Systolic Diastolic

b) Heart Rate #1 beats/min
 #2 beats/min

c) Waist #1 cm → minimal/no clothing
 #2 cm → full clothing

d) Weight kg → minimal/no clothing
 full clothing

e) Hip #1 cm → minimal/no clothing
 #2 cm → full clothing

f) Height cm (without shoes)

42a) Circumference of mid upper right arm: cm

b) Circumference of right calf: cm

c) Head Circumference: cm

d) Upper flexed arm circumference cm

43a) Right arm triceps skinfold: #1 mm
 #2 mm
 #3 mm

b) Right calf skinfold: #1 mm
 #2 mm
 #3 mm

Subject ID

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c) Biceps skinfold

#1 . mm

#2 . mm

#3 mm

d) Subscapular skinfold

#1 . mm

#2 . mm

#3 mm

e) Supra spinal skinfolds

#1 . mm

#2 . mm

#3 mm

44 a) Humerous breadth . cm

b) Femur breadth . cm

45. Grip Strength (Maximal contraction):

a) Non-dominant hand:

#1 kg.

#2 kg.

#3 kg.

b) Dominant hand:

#1 kg.

#2 kg.

#3 kg.

Adult Questionnaire

If subject refuses to provide any of the measures, enter a value of "0" into each of the boxes for that question

For more detailed instructions please refer to the instruction manual

46. Spirometry:

American Thoracic Society criteria for acceptable spirograms:
 Spirograms are acceptable if they are free from:

1. Cough during exhalation
2. Early termination or cut-off
3. Variable effort
4. Leaks
5. Obstructed mouth piece

Subject ID

Centre # Community# Household# Subject#

Subject
 Initials
 F M L

46. Spirometry:

a) FEV1 (Litre): #1 #2 #3

b) Does FEV1 obtained meet ATS criteria?

No → (answer (i) to (iii)) Yes → Go to c)

Reasons for not meeting the ATS criteria: (check all that apply)

- i) Cough
- ii) Values not within 0.2L of each other
- iii) Less than 3 values

c) FVC (Litre): #1 #2 #3

d) Does FVC obtained meet ATS criteria?

No → (answer (i) to (iii)) Yes → Go to e)

Reasons for not meeting the ATS criteria: (check all that apply)

- i) Cough
- ii) Values not within 0.2L of each other
- iii) Less than 3 values

e) PEFR (Litre/min): #1 #2 #3

f) Does PEFR obtained meet ATS criteria?

No → (answer (i) to (ii)) Yes → Go to Q#47

Reasons for not meeting the ATS criteria: (check all that apply)

- i) Cough
- ii) Less than 3 values

Subject ID

Centre # Community# Household# Subject#

Subject
 Initials
 F M L

47. Not applicable in South Africa

48. ECG obtained? No → Go to #49 Yes

a)
 year month day

Place
ECG File
Label Here

b) Please print ECG label #:

49 a) Blood sample obtained? No → Go to #50 Yes

b) Fasting sample Non-fasting sample

c) Time :
 year month day (00:00-23:59) Hours since any food/beverage consumed (excluding water)

d) Please print Blood label #:

Place
Blood label
here

50 a) Urine sample obtained? No → Go to #51 Yes

b) Fasting sample Non-fasting sample

c) Please print Urine label #:

Place
Urine label
here

51. Name of interviewer: _____ Interviewer Code:
 (please print) First Initial Last Name

Attitudes on HIV/AIDS

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39. Mark each answer with a X

J.1	Imagine that a hospital has only one free bed left, and two people with pneumonia need it. The one person is infected with HIV; the other is not infected with HIV. Who should get the bed?	1	The HIV positive person	
		2	The HIV negative person	
		3	It depends / other	
		9	Don't know	

Please respond to the following questions by answering "Yes" or "No".

If you are not sure, chose the "Probably Yes" or "Probably No" response.

If you are quite sure, Choose the "Definitely Yes" or "Definitely No" response.

		Definitely yes 1	Probably yes 2	Probably no 3	Definitely no 4	Don't Know 9
J.2	Do you think the government should provide free health care for people who need it?					
J.3	Do you think the government should provide free health care for people with AIDS?					
J.4	Would it be a good idea for the government to give job training to unemployed young people?					
J.5	Should youth who are infected with HIV get this job training?					
J.6	Should all people who are too sick to work get a welfare grant from the government?					
J.7	Should someone with AIDS who is too sick to work get a welfare grant from the government?					
J.8	Should a woman who got AIDS from sleeping around with many men get this welfare grant from the government?					
J.9	Would you be willing to look after a close family member with AIDS?					
J.10	Imagine that you find out that one of your friends is HIV infected. Would you still be friends with them?					
J.11	Would you drink from the same bottle of water as an HIV infected friend?					
J.12	If you know that a shopkeeper had HIV/AIDS, would you buy fresh vegetables from him or her?					
J.13	Do you think it should be illegal for people with HIV/AIDS to put others at risk of infection through unprotected sex?					
J.14	Do you think people with HIV/AIDS should have to disclose their HIV status to the person they are going to have sex with even if they use a condom?					

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		Definitely yes 1	Probably yes 2	Probably no 3	Definitely no 4	Don't Know 9
J.15	Imagine you meet someone you really like and he/she tells you that he/she is HIV positive, would you still go out on a "date" with him/her?					
J.16	If you loved an HIV positive person, would you have sex with them using a condom?					
J.17	Would you prefer to know who has HIV/AIDS in your community so that you can be careful not to get infected by them?					
J.18	Do you worry that HIV is much easier to catch than we are told?					
J.19	Would you rather not touch someone with HIV/AIDS because you are scared of infection?					
J.20	Do you think the names of people with HIV/AIDS should be made public?					
J.21	Do you think HIV/AIDS is a punishment for sleeping around?					
J.22	Do you think that a school pupil with HIV puts other pupils in their class at risk of infection?					
J.23	Do you think a school pupil with HIV should be allowed to attend school?					
J.24	Do you think that many people who get HIV infected through sex have only themselves to blame?					
J.25	Do you think that some people with HIV/AIDS want to infect other people with the virus?					
J.26	When you hear the word "AIDS" what community or group of people first comes to mind					
Tick only one						
J.27	Between a rich and a poor person: Who is more likely to get HIV/AIDS?	1	A rich person			
		2	A poor person			
		3	Neither / the same			
		9	Don't know			
J.28	Between a black and a white person: Who is more likely to get HIV/AIDS?	1	A black person			
		2	A white person			
		3	Neither / the same / both			
		9	Don't know			
J.29	Between a man and a woman: Who is more likely to get HIV/AIDS?	1	A man			
		2	A woman			
		3	Neither / the same / both			
		9	Don't know			

		Tick only one		
J.30	<i>I am not going to ask you to tell me your result but. Have you ever had an HIV test?</i>	1	Yes	
		2	No	
		8	Refuse	
		9	Don't know	
J.31	Have you heard of any HIV positive people in this area?	1	Yes	
		2	No	
		9	Don't know	
J.32	Do you think people with HIV/AIDS often get treated unfairly or badly by others?	1	Yes	
		2	No	
		9	Don't know	
J.33	Have you met any HIV positive people yourself?	1	Yes	Skip to J.35
		2	No	
		9	Don't know	
J.34	If yes: What is your relationship with this person or people? Interviewer: do not read list. You can tick more than one	1	Partner (Husband/wife, boyfriend, girlfriend)	
		2	Sister/brother	
		3	Parent	
		4	Other relative	
		5	Friend	
		6	Neighbour	
		7	Classmate	
		8	Colleague at work/	
		9	Other / none of the above	
		10.	Refuse to answer	
J.35	If you know you were infected with the HIV virus, would you keep it a secret from most people?	1	Yes	
		2	No	
		9	Don't know	
J.36	If you told someone, who would you tell? Interviewer: do not read list; multi-mentions possible You can tick more than one	1	Partner (Husband/wife, boyfriend, girlfriend)	
		2	Sister/brother	
		3	Parent	
		4	Other relative	
		5	Friend	
		6	Neighbour	
		7	Priest / someone in my church	
		8	School teacher	
		10	Anyone	
		11	Other	
		99	Don't know	
J.37	In your opinion, how at risk are you of HIV infection? Interviewer: read list Tick only one	1	No risk	
		2	Very small risk	
		3	Some risk	
		4	Great risk	
		9	Don't know	

J.38	Imagine you had unprotected sex yesterday. Would you be more afraid of pregnancy or of infection with HIV? Interviewer: do not read list Tick only one	1	More afraid of pregnancy	
		2	More afraid of HIV infection	
		3	Afraid of both pregnancy and infection	
		4.	Afraid of neither	
		9	Don't know	
J.39	Who do you think is more likely to get HIV: someone with <u>one</u> sexual partner who does <u>not</u> use condoms or someone with <u>many</u> partners who <u>always</u> uses condoms? Interviewer: Tick only one	1	A person with one partner who does not use condoms.	
		2	A person with many partners who always uses condoms.	
		3	They are at equal risk	
		9	Don't Know	

Adult Questionnaire

40. Cause of death

- 1= Heart disease
- 2= Stroke
- 3= TB
- 4= Cancer
- 5= HIV
- 6= Injury
- 7= Other
- 8= Unknown

Cancer Sites

- 1= Mouth
- 2= Esophagus
- 3= Stomach
- 4= Small intestine
- 5= Large intestine including rectum
- 6= Pancreas
- 7= Liver
- 8= Lung
- 9= Breast
- 10= Cervical/uterine/ovarian
- 11= Prostate
- 12= Head and neck
- 13= Other

Date of death:

When completing the date of death, enter actual year and month of death.

Example: If a respondent indicates that the date of death was March 2004, enter as follows

Is this member alive? No Yes →

0	4	Year	0	3	Month
---	---	------	---	---	-------

If exact month is not known, please obtain an approximate guess

PURE

Adult Questionnaire

Subject ID

Centre # Community# Household # Subject #

Subject Initials
 F M L

40. Details on all members (enumerated at baseline) in the household:

Member # Name _____ Is this member a participant? No Yes

Is this member alive? No → Complete details below Yes → Go to Next Member #

If No, indicate cause of death: Date of death: YR Mo

If Cause of death =4, please indicate site If Cause of death =7, please specify, _____

Member # Name _____ Is this member a participant? No Yes

Is this member alive? No → Complete details below Yes → Go to Next Member #

If No, indicate cause of death: Date of death: YR Mo

If Cause of death =4, please indicate site If Cause of death =7, please specify, _____

Member # Name _____ Is this member a participant? No Yes

Is this member alive? No → Complete details below Yes → Go to Next Member #

If No, indicate cause of death: Date of death: YR Mo

If Cause of death =4, please indicate site If Cause of death =7, please specify, _____

Member # Name _____ Is this member a participant? No Yes

Is this member alive? No → Complete details below Yes → Go to Next Member #

If No, indicate cause of death: Date of death: YR Mo

If Cause of death =4, please indicate site If Cause of death =7, please specify, _____

Adult Questionnaire

40. Cause of death

- 1= Heart disease
- 2= Stroke
- 3= TB
- 4= Cancer
- 5= HIV
- 6= Injury
- 7= Other
- 8= Unknown

Cancer Sites

- 1= Mouth
- 2= Esophagus
- 3= Stomach
- 4= Small intestine
- 5= Large intestine including rectum
- 6= Pancreas
- 7= Liver
- 8= Lung
- 9= Breast
- 10= Cervical/uterine/ovarian
- 11= Prostate
- 12= Head and neck
- 13= Other

Date of death:

When completing the date of death, enter actual year and month of death.

Example: If a respondent indicates that the date of death was March 2004, enter as follows

Is this member alive? No Yes →

Year	Month
0 4	0 3

If exact month is not known, please obtain an approximate guess

PURE

Adult Questionnaire

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Subject ID

Centre # Community# Household # Subject #

Subject Initials
 F M L

40. Details on all members (enumerated at baseline) in the household:

Member # Name Is this member a participant? No Yes

Is this member alive? No → Complete details below Yes → Go to Next Member #

If No, indicate cause of death: Date of death: YR Mo

If Cause of death =4, please indicate site If Cause of death =7, please specify,

Name of interviewer: _____ Date
 (Please print) Last Name First Initial year month day

ADDENDUM D
PHYSICAL ACTIVITY QUESTIONNAIRE

PURE-SA Project (Prospective Urban and Rural Epidemiology)

Physical activity questionnaire

Date: _____ Place: _____ Interviewer: _____
The information on this questionnaire is confidential

1. Subject number _____ (1-4)
2. Gender

Male	1	Female	2
------	---	--------	---

 (5)
3. What is your main occupation?.....

Low level: office work, housework, scholar	1
Middle level: factory work, carpentry, farming, hospital nurse, plumber	2
High level ("sweat work"): construction work, digging, manual labour	3

 (6)
4. At work I sit

1. never	2. seldom	3. sometimes	4. often	5. always
----------	-----------	--------------	----------	-----------

 (7)
5. At work I stand

1. never	2. seldom	3. sometimes	4. often	5. always
----------	-----------	--------------	----------	-----------

 (8)
6. At work I walk

1. never	2. seldom	3. sometimes	4. often	5. always
----------	-----------	--------------	----------	-----------

 (9)
7. At work I lift heavy loads

1. never	2. seldom	3. sometimes	4. often	5. always
----------	-----------	--------------	----------	-----------

 (10)
8. At work I am tired

1. never	2. seldom	3. sometimes	4. often	5. always
----------	-----------	--------------	----------	-----------

 (11)
9. At work I sweat

1. never	2. seldom	3. sometimes	4. often	5. always
----------	-----------	--------------	----------	-----------

 (12)
10. If you work away from home, how do you get to work/school?

walk	1
cycle	2
car/taxi	3

 (13)
11. How long does it take you to walk/cycle to work/school? (or to the taxi rank/ bus stop/ train station)

0-15 min	1
16-30 min	2
31-60 min	3
1-2 hours	4

 (14)
12. If you walk or cycle to work/school, what is your usual pace? (or to taxi rank/bus stop/ train station)

casual strolling	1
fairly brisk	2
brisk/fast	3

 (15)
13. Do you climb stairs often?

yes	1
no	2

 (16)
14. If yes, how many flights of stairs do you climb each day? (1 flight = 10 steps) (17)
15. How many days per week do you climb steps? (18)
16. Do you play sport?

yes	1
no	2

 (19)
17. Which sport do you play most frequently?

low level: bowling, golf, billiards	1	0.76*
middle level: tennis, athletics, cycling	2	1.26
high level: soccer, rugby, netball, boxing	3	1.76(20)

 (21-23)
18. How many hours per week do you practice? <1/ 1-2/ 2-3/ 3-4/ >4 (Write appropriate code in space) (24-26)
19. How many months per year? (Write appropriate code in space) (24-26)

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



20. If you play a second sport, which is it?

low level: bowling, golf, billiards	1	0.76*
middle level: tennis, athletics, cycling	2	1.26
high level: soccer, rugby, netball, boxing	3	1.76(27)

 (28-30)
21. How many hours per week do you practice? <1/ 1-2/ 2-3/ 3-4/ >4

0.5, 1.5, 2.5, 3.5, 4.5*		
<1/ 1-3/ 4-6/ 7-9/ >9		

 (31-33)
22. How many months per year? 0.04, 0.17, 0.42, 0.67, 0.92*³

--	--	--	--	--

 (31-33)
23. During leisure time I watch TV/ do sitting activities (read, needle-work, play cards)

1. never	2. sel-dom	3. some-times	4. often	5. al-ways
----------	------------	---------------	----------	------------

 (34)
24. During leisure time I walk/ do standing activities (gardening, housework)

1. never	2. sel-dom	3. some-times	4. often	5. al-ways
----------	------------	---------------	----------	------------

 (35)
25. Other leisure-time activities:..... (leisure-time = time off from work/ school)

	2. sel-dom	3. some-times	4. often	5. al-ways
--	------------	---------------	----------	------------

 (36)



ADDENDUM E

METHODOLOGY OF THE SPECTROLYSE® PAI-1 ACTIVITY ASSAY

**(Sekisui Diagnostics, LLC, Stamford, Connecticut, USA) to
determine PAI-1_{act}**

Step 1: Preparation and storage of reagents

Prepare the reagents in the following sequence: t-PA / PAI-1 depleted plasma, the 6000 IU/ml t-PA containing solution, working strength Imidazole buffer solution and lastly the 40 IU/ml t-PA containing solution. The plasminogen activator reagent (PAR) is prepared only 15 minutes before it is used.

Imidazole buffer (pH 7.2, 10 × concentrated, 3 ml)

1. Prepare a working strength solution of the buffer by diluting the buffer concentrate (3 ml) with filtered deionized / distilled (dd) water (27 ml) using a ration of 1:10
2. Final working strength Imidazole buffer solution is 30 ml
3. Stable for 1 month at 2 - 8°C

Plasminogen activator reagent (PAR)

1. Reconstitute the vial with 6.25 ml of working strength Imidazole buffer
2. Vortex vigorously for 1 minute
3. Let it stand on ice for 15 minutes
4. Stable for 24 hours at 2 - 8°C

Single-chain human t-PA

- **Reconstitute (solution contains 6000 IU/ml t-PA)**
 1. Reconstitute the vial with 1 ml acetate buffer (included in kit)
 2. Mix gently and place on ice
 3. Can be used for up to 4 days when stored at 2 - 8°C
- **To obtain a solution containing 40 IU/ml t-PA**
 1. Add 50 µL of the 6000 IU/ml t-PA to 7.5 ml of the working strength Imidazole buffer
 2. Stable for 24 hours at 2 - 8°C

t-PA / PAI-1 depleted plasma (1ml, lyophilized)

1. Reconstitute the vial with 1 ml filtered dd water
2. Mix gently and allow to stand for 15 minutes for complete dissolution
3. Stable for 2 weeks at -20°C

Acetate buffer (pH 3.9, 7 ml)

1. Ready to use
2. Stable for 1 year at 2 - 8°C

Stop reagent (4ml)

1. Ready to use
2. Stable for 1 year at 2 - 8°C

Step 2: Preparation of PAI-1 standards

Different PAI-1 standards are prepared as indicated below by adding the different constituents together:

0 IU/ml PAI-1 standard

125 µL t-PA / PAI-1 depleted plasma

125 µL 40 IU/ml t-PA solution

Incubate for 15 min at 25°C

40 IU/ml PAI-1 standard

125 µL t-PA / PAI-1 depleted plasma

125 µL working strength Imidazole buffer

Incubate for 15 min at 25°C

10 IU/ml PAI-1 standard

25 µL 40 IU/ml PAI-1 standard

75 µL 0 IU/ml PAI-1 standard

20 IU/ml PAI-1 standard

50 µL 40 IU/ml PAI-1 standard

50 µL 0 IU/ml PAI-1 standard

30 IU/ml PAI-1 standard

75 µL 40 IU/ml PAI-1 standard

25 µL 0 IU/ml PAI-1 standard

Step 3: Reconstitution of controls

The TriniLIZE Fibrinolysis Reference Plasma kit (Tcoag, Ireland) was used as a control to verify the performance and accuracy of the Spectrolyse® PAI-1 Activity Assay in order to ensure quality control. When this control is used in conjunction with a laboratory test determining PAI-1 activity, the recommended range for PAI-activity is 8.3 – 12.4 IU/ml.

1. Ensure that the vial reaches room temperature before opening
2. Add 0.5 ml of filtered dd water to a vial containing 0.5 ml of the control plasma
3. Gently agitate for 5 minutes until completely dissolved
4. Use within 30 minutes

Step 4: Preparation of plasma samples and controls

1. Frozen plasma should be thawed at 37°C
2. Dilute each plasma sample by using 50 µL of each plasma sample and adding 50 µL of the 40 IU/ml t-PA solution to it
3. To prepare the controls, take 50 µL of the control and add 50 µL of the 40 IU/ml t-PA solution to it
4. Incubate for 15 min at 25°C to allow for the t-PA and PAI-1 to react
5. Prepare the plasma samples and controls at the same time as the PAI-1 standards

Step 5: Incubation of PAI-1 standards, plasma samples and controls

1. Add 100 µL of the acetate buffer to each of the tubes containing the standards, plasma samples and controls and mix
2. Incubate for 20 minutes in a 37°C water bath
3. Add 2 ml of filtered dd water and mix

Step 6: Determination of PAI-1 activity

1. The outer microwells of the microwell plate is not used in order to ensure uniform temperature distribution throughout the plate
2. Fill the unused outer microwells with dd water
3. Filling of the 60 microwells:
 - PAI-1 standards: fill 2 microwells with 20 µL each of the 5 prepared PAI-1 standards (total of 10 microwells)
 - Plasma samples: fill 47 microwells with the prepared samples (40 IU/ml t-PA solution diluted plasma) by adding 20 µL of each sample to a microwell

- Controls: fill 3 microwells with the prepared controls (40 IU/ml t-PA solution diluted controls) by adding 20 μ L of the control to each of the 3 microwells
- 4. Add 200 μ L of ice cold PAR to standards, plasma samples and controls
- 5. Seal the plate with an acetate cover sheet
- 6. Incubate by floating the plate in a water bath at 37°C for 90 minutes
- 7. Remove the plate from the water bath
- 8. Add 50 μ L stop reagent to each microwell
- 9. Measure the solution absorbance at 405 nm by using a spectrophotometer (Thermo Scientific, Multiskan FC)
- 10. Prepare a polynomial calibration curve from the absorbance results for each microwell plate by plotting the absorbance 405 nm for each PAI-1 standard against its corresponding concentration.
- 11. Interpolate the PAI-1_{act} of the plasma sample directly from the standard curve.

ADDENDUM F
PLASMA FIBRINOLYTIC POTENTIAL PROTOCOL

1. Start the microplate reader (Labsystems, Multiskan Ascent, model no. 354) and set the temperature at 37 °C.
2. Frozen plasma should be thawed for 5 min at 37 °C in a water bath.
3. Prepare 1400 µl of the assay mixture that consists of the following stock solutions: 149.3 µl tissue factor (Dade Innovin, Siemens Healthcare Diagnostics Inc., Marburg, Germany), 373.4 µl CaCl₂, 93.4 µl t-PA (Actilyse, Boehringer-Ingelheim, Ingelheim, Germany), 186.6 µl phospholipid vesicles (Phospholipids-TGT, Rossix, Mölndal, Sweden) and 597.3 µl assay buffer (ingredients: HEPES, NaCl, KCl, 1% BSA (pH 7.4)) at room temperature.
4. Dilute plasma samples at room temperature: add 71.4 µl of citrated plasma sample to each well of a microtiter plate, three wells on each plate are used as controls (add 71.4 µl citrated pool plasma); thereafter add 50.0 µl of assay buffer to each well using a multi-channel pipette; shake on a plate shaker for 10 seconds at 1400 rpm. We did not use the two outer columns of the 96 well plates.
5. Add 15 µl of the assay mixture to each well of another microtiter plate (NUNC-Maxisorp). We did not use the two outer columns of the 96 well plates and filled these columns with dd water to ensure even temperature distribution throughout the plates.
6. Using a multi-channel pipette, pipette 85 µl of the diluted plasma samples over to the plate that contains the assay mixture. These steps are performed quickly and the time from adding the plasma dilution to the assay mix up until the measurements start (step 9), is noted as the reaction starts as soon as the plasma is pipetted into the assay mix.
7. Shake the plate on a plate shaker 10 seconds at 1400 rpm.
8. Using wide-bore tips add 50 µl liquid paraffin oil (Merck) to each well to cover it and prevent evaporation during measurement.

9. Place the plate into the plate reader. Start measuring the absorbance at 405 nm every 9 seconds for the first two minutes, every 15 seconds up to 30 minutes and thereafter every minute until the clots have broken down or up to 270 minutes.