The association between alcohol consumption, PAI-1 activity and fibrinogen concentration in black South Africans

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ABSTRACT

INTRODUCTION AND AIM

The prevalence of cardiovascular disease (CVD) is increasing in the black South African population. At increased levels, plasminogen activator inhibitor type-1 (PAI-1) and fibrinogen, which are two of the best known haemostatic risk factors, may increase the risk of CVD. Fibrinogen concentrations have been shown to be higher, and PAI-1 levels *to be* significantly lower, in black South African populations than in Caucasians. Alcohol consumption has been shown to influence the risk of CVD, amongst others, through effects on haemostasis. Cross-sectional epidemiological studies indicate that moderate amounts of alcohol seem to correlate negatively with fibrinogen, while PAI-1 levels seem to increase with heavy alcohol consumption. However, these studies were conducted in Caucasian populations and, owing to differences between black Africans and Caucasians in absolute fibrinogen and PAI-1 levels, the question arises whether these risk factors will associate with habitual alcohol consumption in black Africans in the same way as they do in Caucasians. In the present study, we investigated the association between alcohol consumption, fibrinogen concentration and $PAI\text{-}1_{act}$, as well as the influence of gender, urbanisation, waist circumference, body mass index (BMI), triglyceride concentration and the 4G/5G polymorphism (the last two for PAI-1) on this association in the South African Prospective Urban and Rural Epidemiological (PURE) study population.

PARTICIPANTS AND METHODS

Approximately 1000 rural and 1000 urban, apparently healthy, black men and women aged 35-60 years participated in the South African arm of the international PURE study. Over a twelve-week period in 2005, habitual alcohol consumption (g/day) was determined by quantitative food frequency questionnaires administered by trained fieldworkers, and blood samples and anthropometrical measurements were collected.

RESULTS

Heavy alcohol consumption was associated with an increase in PAI-1_{act} in the total population after adjustment for triglycerides and waist circumference. In participants with increased triglyceride concentrations (≥ 1.7 mmol/l) and in abdominally obese and obese (BMI ≥ 30 kg/m^2) participants who drank heavily, PAI-1_{act} was significantly higher than in non-drinkers. This alcohol-related increase in PAI-1_{act} was not observed, however, in individuals with normal waist circumference measurements or in individuals with normal triglyceride concentrations. In the total population, moderate alcohol consumption was associated with a decrease in fibrinogen concentration, compared with non-drinkers, and reached a plateau with heavy alcohol consumption. This association was also seen in participants with normal waist circumference and BMI, as well as in overweight participants. However, in abdominally obese participants and those with a BMI of more than 30 kg/m^2, the consumption of moderate amounts of alcohol was not associated with a decrease in fibrinogen concentrations. Neither gender, the 4G/5G polymorphism (PAI-1 only) nor urbanisation significantly influenced the associations between alcohol consumption and fibrinogen or PAI-1_{act}.

CONCLUSION

Despite the finding that fibrinogen concentration is generally higher, and PAI-1_{act} lower, in black South Africans than in Caucasians, the association between these two haemostatic risk factors and alcohol consumption seems to follow the same pattern as in Caucasian populations. Heavy alcohol consumption was associated with an increase in PAI-1_{act}, while moderate alcohol consumption was associated with a decrease in fibrinogen concentration, which was not further decreased in the heavy alcohol consumers. Normal triglyceride concentrations and waist circumference, however, seem to protect against the alcohol-related PAI-1_{act} increase in this black African population.

OPSOMMING

Die assosiasie tussen alkoholinname, PAI-1-aktiwiteit en fibrinogeenkonsentrasie in swart Suid-Afrikaners

INLEIDING EN DOEL

In die swart Suid-Afrikaanse bevolking neem die voorkoms van kardiovaskulêre siekte (KVS) toe. Plasminogeenaktiveerderinhibeerder tipe-1 (PAI-1) en fibrinogeen is twee van die mees bekende hemostatiese risikofaktore, en kan by verhoogde vlakke die risiko vir KVS verhoog. Hoër fibrinogeen-, en laer PAI-1-vlakke as in blanke populasies is in swart Suid-Afrikaners gevind. Alkoholinname kan die risiko van KVS beïnvloed deur, onder andere, die effek daarvan op hemostase. Epidemiologiese dwarsdeursnit studies dui dat die inname van matige hoeveelhede alkohol 'n negatiewe assosiasie met fibrinogeenkonsentrasie toon, terwyl dit blyk dat PAI-1 verhoog met hoë vlakke van alkoholinname. Die resultate van bogenoemde studies is egter van blanke populasies en die verskil in absolute fibrinogeen- en PAI-1- vlakke tussen swart Suid-Afrikaners en blanke populasies laat die vraag ontstaan of hierdie risikofaktore in swart Suid-Afrikaners op dieselfde manier met alkoholinname geassosieer sal wees as in blanke populasies. In die huidige studie is die assosiasie tussen alkoholinname, fibrinogeenkonsentrasie en PAI-1-aktiwiteit, asook die invloed van geslag, verstedeliking, middelomtrek, ligaamsmassa-indeks (LMI), trigliseriedkonsentrasie en die 4G/5G polimorfisme (laasgenoemde twee net vir PAI-1) op hierdie assosiasie in die Suid-Afrikaanse *Prospective Urban and Rural Epidemiological* (PURE) studiepopulasie bepaal.

STUDIEPOPULASIE EN METODES

Op die oog af gesonde, swart mans en vroue tussen die ouderdomme van 35 en 60 jaar, waarvan ongeveer 1000 van verafgeleë en 1000 van stedelike gebiede afkomstig was, het aan die Suid-Afrikaanse been van die internasionale PURE studie deelgeneem. Bloedmonsters, antropometriese metings en gewoontelike alkoholinname (g/dag) deur opgeleide veldwerkers met die hulp van kwantitatiewe voedselfrekwensievraelyste is in 2005 oor 'n tydperk van twaalf weke versamel.

RESULTATE

Nadat daar gekontroleer is vir die effek van middelomtrek en trigliseriede, was hoë vlakke van alkoholinname in hierdie populasie geassosieer met hoër PAI-1-aktiwiteit ($PAI\text{-}1_{akt}$). $PAI\text{-}1_{akt}$ van abdominaalvetsugtige, vetsugtige (LMI ≥ 30 kg/m^2) asook proefpersone met verhoogde trigliseriedkonsentrasies (≥ 1.7 mmol/l) wat hoë vlakke van alkohol ingeneem het, was betekenisvol hoër as die van die nie-drinkers. Hierdie alkoholverwante verhoging in $PAI\text{-}1_{akt}$ is nie gesien in proefpersone met normale middelomtrekmetings of in proefpersone met normale trigliseriedkonsentrasies nie. Matige alkoholinname was in die totale PURE populasie geassosieer met laer fibrinogeenkonsentrasies as in nie-drinkers, en het met hoë vlakke van alkoholinname 'n plato bereik. Hierdie assosiasie is in proefpersone met normale middelomtrekmetings en LMI, sowel as in oorgewig proefpersone gesien. Matige alkoholinname was in proefpersone wat abdominaal vetsugtig was, of 'n LMI van meer as 30 kg/m^2 gehad het nie geassosieer met 'n verlaging in fibrinogeenkonsentrasie nie. Nie geslag, die 4G/5G- polimorfisme (slegs vir PAI-1) of verstedeliking het die assosiasie tussen alkoholinname, fibrinogeen en $PAI\text{-}1_{akt}$ betekenisvol beïnvloed nie.

SAMEVATTING

Ten spyte van fibrinogeenkonsentrasies wat oor die algemeen hoër, en $PAI\text{-}1_{akt}$ wat laer was in swart Suid-Afrikaners as in blanke populasies, lyk dit tog of die assosiasie tussen hierdie twee hemostatiese risikofaktore en alkoholinname dieselfde is as in blanke populasies. Hoë vlakke van alkoholinname was geassosieer met verhoogde PAI-1-vlakke, terwyl matige alkoholinname geassosieer was met 'n verlaging in fibrinogeenkonsentrasie. Fibrinogeen was nie verder verlaag in proefpersone wat hoë vlakke van alkohol ingeneem het nie. Dit blyk dat normale trigliseriedkonsentrasies en middelomtrekmetings moontlik hierdie swart populasie teen die alkoholverwante verhoging in $PAI\text{-}1_{akt}$ kan beskerm.

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LIST OF ABBREVIATIONS

Acetyl CoA	Acetyl co-enzyme A
ADH	Alcohol dehydrogenase
AIDS	Acquired immunodeficiency syndrome
ALDH	Acetaldehyde dehydrogenase
ANCOVA	Analysis of co-variance
ANOVA	Analysis of variance
BAC	Blood alcohol concentration
BMI	Body mass index
°C	Degrees Celsius
%CDT	Percentage carbohydrate-deficient transferrin
CAD	Coronary artery disease
CDT	Carbohydrate-deficient transferrin
CHD	Coronary heart disease
CI	Confidence interval
cm	Centimeters
CO2	Carbon dioxide
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
f	Frequency (number of subjects per group)

FAEE	Fatty acid ethyl ester
FAS	Foetal alcohol syndrome
FDP	Fibrin degradation products
g	gram
g	Gravitational force
g/day	Gram per day
g/l	Gram per litre
g/week	Gram per week
GGT	Gamma glutamyl transferase
HDL-chol	High density lipoprotein cholesterol
HF	High fat diet
HIV	Human immunodeficiency virus
IDT	Integrated DNA technologies
IL-1β	Interleukin-1beta
IL-6	Interleukin-6
KDa	Kilo Dalton
LDL-chol	Low density lipoprotein cholesterol
MD	Mediterranean diet
MEOS	Microsomal ethanol oxidizing system
mg	milligram
mg/dl	Milligram per decilitre
MI	Myocardial infarction
ml	millilitre

mmol/l	Millimol per litre
mRNA	Messenger ribonucleic acid
n	Size of total study population
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NCBI	National centre for biotechnology information
ng/l	Nanogram per litre
ng/ml	Nanogram per millilitre
PAI-1	Plasminogen activator inhibitor type-1
$PAI\text{-}1_{act}$	Plasminogen activator inhibitor type-1 activity
PCR	Polymerase chain reaction
PEt	Phosphatidyl ethanol
PLD	Phospholipase D
PURE study	Prospective urban and rural epidemiological study
QFFQ	Quantitative food frequency questionnaire
SBP	Systolic blood pressure
SD	Standard deviation
SMAC	Sequential multiple analyzer computer
SNP	Single nucleotide polymorphism
TAFI	Thrombin activatable fibrinolysis inhibitor
TCA cycle	Tricarboxilic acid cycle

TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TGF-β	Transforming growth factor beta
THUSA	Transition and health during urbanisation of South Africans
TNF-α	Tumor necrosis factor alpha
t-PA	Tissue plasminogen activator
U/l	Units per litre
U/ml	Units per millilitre
VLDL	Very-low-density lipoprotein
v	Versus
VSMC	Vascular smooth muscle cells
vWF	Von Willebrand factor
WC	Waist circumference
yr	Year
µg/l	Microgram per litre

CHAPTER 1: INTRODUCTION

1.1. BACKGROUND

In recent decades the prevalence of non-communicable diseases, including cardiovascular disease (CVD), has increased in both developed and developing countries (Yusuf *et al.*, 2001; Deeg *et al.*, 2008; Teo *et al.*, 2009). It is predicted that the overall burden of CVD will rise by approximately 150% in developing countries in the next 20 years (Murray & Lopez, 1996). In South Africa too, the black African population is experiencing an increased prevalence of CVD (Sliwa *et al.,* 2008). This is probably attributable to a significant health transition due to urbanisation (Vorster, 2002).

The modifiable risk factors for CVD in Caucasians include hypertension, hypercholesterolaemia, low levels of high-density lipoprotein cholesterol (HDL-chol), cigarette smoking, diabetes mellitus, obesity, physical inactivity, hypertriglyceridaemia, unwise dietary choices and heavy alcohol consumption (Akinboboye *et al.*, 2003; Yusuf *et al.*, 2004; Amira *et al.*, 2006). Apart from these classical risk factors, haemostatic variables, fibrinogen and plasminogen activator inhibitor type-1 (PAI-1) are now also considered to be independent risk factors for CVD (Vorster *et al.*, 1998; Kamath & Lip, 2003; Koenig, 2003; Hawkins, 2004). Elevated concentrations of fibrinogen may be involved in thrombosis through its role as the substrate for thrombin in the coagulation cascade and the role it plays in platelet aggregation (Koenig, 2003). Formed clots are broken down through the process of fibrinolysis, which is inhibited by PAI-1, which binds tissue plasminogen activator (t-PA). Therefore, elevated levels of PAI-1 will result in more t-PA being bound and the fibrinolytic process being inhibited even further, which may be associated with CVD (Kohler & Grant, 2000; Hawkins, 2004). In general, higher levels of fibrinogen and significantly lower levels of PAI-1 have been observed in black South Africans, compared with levels typically observed in healthy Caucasians, but the reason for this is not yet fully understood (Jerling *et al.*, 1994; Vorster *et al.*, 1998; Vorster, 2002; Greyling *et al.*, 2007; Pieters & Vorster, 2008).

The association between alcohol consumption and CVD risk can be described as being U- or J-shaped, with very low and excessive amounts of alcohol intake resulting in increased CVD risk, whereas the ingestion of moderate amounts of alcohol on a regular basis results in a decreased risk of CVD (Kiechl *et al.*, 1998; Salem & Laposata, 2005). The factors contributing to this protective effect of moderate alcohol consumption are not yet fully understood, but possible mechanisms include elevated levels of HDL-chol or the inhibitory effect of alcohol on platelet aggregation (Kiechl *et al.*, 1998; Salem & Laposata, 2005). Alcohol is also known to influence haemostatic variables, although its effect on these variables is less clear. It seems that moderate intake of alcohol can decrease concentrations of clotting factors like fibrinogen, while high alcohol intakes may lead to decreased fibrinolysis due to increased PAI-1 levels (Salem & Laposata, 2005). The above-mentioned influence of alcohol on PAI-1 and fibrinogen levels was observed in Caucasian participants, however, and very little information is available regarding the effect of alcohol on PAI-1 and fibrinogen in black populations.

The question that now arises is whether moderate alcohol consumption will also be related to decreased fibrinogen concentrations in this black South African population, in which the healthy present with fibrinogen concentrations that are higher than those typically seen in Caucasians. Also, because PAI-1 levels of black South Africans are significantly lower than levels typically observed in Caucasians, it remains to be determined whether high levels of alcohol consumption will also be related to increased PAI-1 levels in this population, as is the case in Caucasian populations.

There is still a lack of sufficient data related to habitual alcohol consumption, PAI-1 and fibrinogen levels in black South Africans, as well as to the associations between these factors. As a result of the epidemiological transition in this population, further research is needed to monitor the changing trend in CVD risk factors. The Prospective Urban and Rural Epidemiology (PURE) study is an international cohort study, and, with the South African baseline data of 2010 apparently healthy black African participants collected in 2005, it will be possible to investigate all the issues raised.

1.2. AIM AND OBJECTIVES

The main aim of this study was to determine the association between habitual alcohol consumption, PAI-1 activity and fibrinogen concentration in the South African PURE population.

In order to achieve the above-mentioned aim, the baseline data for the PURE population (n = 2010), collected in 2005, were used in the present cross-sectional study. Habitual alcohol intakes obtained from quantitative food frequency questionnaires (QFFQ) were related to fibrinogen concentration and PAI-1_{act}. The specific objectives were:

- To investigate whether GGT (gamma glutamyl transferase) and %CDT (percentage carbohydrate-deficient transferrin) can be used as proxy markers to indicate the association between alcohol, PAI-1_{act} and fibrinogen concentration. Since alcohol consumption is self-reported, it may not reflect actual alcohol consumption accurately. GGT and %CDT are considered to be proxy (biological) markers that can be used to reflect chronic excessive alcohol consumption.
- To determine the association of habitual alcohol consumption with fibrinogen concentrations and PAI-1_{act}.
- To investigate the effect of urbanisation on the association between alcohol consumption, fibrinogen concentration and PAI-1_{act}. Many factors associated with urbanisation may themselves affect PAI-1_{act} and fibrinogen, and therefore their relationship with alcohol.
- To investigate the association between habitual alcohol consumption and PAI-1_{act} in relation to the 4G/5G polymorphism, since this polymorphism is considered to be a response polymorphism.
- To investigate the association between habitual alcohol consumption and fibrinogen concentration and PAI-1_{act} in relation to body composition (waist circumference and body mass index). Body composition is affected by alcohol consumption and body composition itself can affect fibrinogen concentration and PAI-1_{act}.

- To investigate whether the association between habitual alcohol consumption and $PAI\text{-}1_{act}$ is modulated by triglyceride concentration.

1.3. RESEARCH TEAM

Title, initials and surname	Affiliation	Role in the study
Prof. A. Kruger	Africa Unit for Transdisciplinary Health Research, North-West University, Potchefstroom Campus	South African PURE study coordinator.
Prof. M Pieters	TReNDS Centre of Excellence – Nutrition, North-West University, Potchefstroom Campus	Supervisor of M.Sc. dissertation, guidance regarding protocol writing, statistical analysis, interpretation of results and writing up of the data.
Dr. T. Hoekstra	Julius Centre for Health Sciences and Primary Care, University Medical Centre, Utrecht, the Netherlands	Co-supervisor of M.Sc. dissertation, guidance regarding protocol writing, statistical analysis, interpretation of results and writing up of the data
Prof. J.C. Jerling	TReNDS Centre of Excellence – Nutrition, North-West University, Potchefstroom Campus	Assistant supervisor of M.Sc. dissertation, guidance regarding writing up of the data
Prof. S. Ellis	Statistical Consultation Services, North-West University, Potchefstroom Campus	Guidance regarding statistical analyses
Miss. Z. de Lange	TReNDS Centre of Excellence – Nutrition, North-West University, Potchefstroom Campus	Full-time M.Sc. student, protocol writing, statistical analysis, interpretation of results and writing up of the literature and data

1.4. STRUCTURE OF THIS DISSERTATION

This dissertation is presented in chapter format. It was technically edited in the style required by the North-West University, and has been edited by a competent language editor. This introductory chapter is followed by Chapter 2, in which a review of the literature is given. This review includes the metabolism of alcohol, information regarding the use of GGT and %CDT to determine alcohol abuse, and the effects of alcohol consumption on health. An overview of coagulation and fibrinolysis as well as of the roles of PAI-1 and fibrinogen in CVD is presented, and a synopsis given of findings from cross-sectional epidemiological and intervention studies on the effects of alcohol on these two haemostatic risk factors. This is followed by a survey of drinking patterns of black South Africans and of differences in PAI-1 and fibrinogen levels between black South Africans and Caucasians.

Chapter 3 describes the study design of the PURE study, the recruitment and characteristics of the participants, as well as experimental methods used, which include anthropometric measurements, dietary intake analysis, blood sampling and analysis and statistical methods.

In Chapter 4, the results of the present study are presented. This chapter includes the baseline characteristics of the PURE study population, and population characteristics of the different drinking categories. Participants were divided into drinking categories as follows: participants who reported consuming no alcohol were classified as non-drinkers; men who consumed less than 30 g (approximately 2 units per day) and women who consumed less than 15 g (approximately 1 unit per day) alcohol per day, were classified as moderate drinkers; the category of heavy drinkers was constituted of women who drank 15 g alcohol and more, and men who drank 30 g alcohol and more per day. The association between alcohol, PAI-1_{act} and fibrinogen, as well as the influences of various population characteristics on the association of alcohol consumption and PAI-1_{act} and fibrinogen, are presented in Tables 4.3 and 4.4.

In Chapter 5, the results of the present study are discussed, compared with the available literature and possible reasons for the results obtained are given. Although not the main focus of this dissertation, the influence of gender, urbanisation, waist circumference and BMI on PAI-1_{act} and fibrinogen, as well as of triglyceride concentration and the 4G/5G polymorphism on PAI-1_{act}, are discussed in sections 5.2 and 5.3, after which the association between alcohol consumption, PAI-1_{act} and fibrinogen, as well as the influence of the above-mentioned factors, if any, on these associations, are discussed in sections 5.4 and 5.5. This is followed by a review of the limitations of this study, the conclusion, and recommendations for future research.

CHAPTER 2: LITERATURE REVIEW

2.1. INTRODUCTION

In the developing world, there is an increase in classical risk factors linked to cardiovascular disease (CVD), such as smoking, elevated levels of low-density lipoprotein cholesterol (LDL-chol), low levels of high-density lipoprotein cholesterol (HDL-chol), high blood pressure, elevated glucose, physical inactivity and obesity, as well as rates of CVD (Yusuf *et al.*, 2001). Two of the best known haemostatic cardiovascular risk markers, plasminogen activator inhibitor type-1 (PAI-1) and fibrinogen, may, at increased plasma levels, sway the haemostatic balance between clot formation and breakdown (fibrinolysis) in favour of clot formation, leading to the development of cardiovascular disease (Mertens & Van Gaal, 2002). Alcohol has been shown to influence these two haemostatic risk factors.

The majority of the literature on alcohol and PAI-1 shows that moderate amounts of alcohol have no effect on PAI-1, and that heavy alcohol consumption leads to increased PAI-1 levels (Marques-Vidal *et al.,* 1995; Djousse *et al.,* 2000; Yarnell *et al.,* 2000; Mukamal *et al.,* 2001; Sasaki *et al.,* 2001). Epidemiological studies show a negative correlation between alcohol consumption and fibrinogen (Meade *et al.,* 1979; Folsom *et al.,* 1991; Lee *et al.,* 1995; Marques-Vidal *et al.*, 1995; Yarnell *et al.,* 2000; Wannamethee *et al.,* 2003; Mukamal *et al.*, 2004; Schröder *et al.,* 2005; Perissinotto *et al.,* 2009; Tolstrup *et al.,* 2009). Some studies found, however, that fibrinogen concentrations reached a plateau or increased with heavy alcohol consumption (Krobot *et al.,* 1992; Mennen *et al.,* 1999a; Mukamal *et al.*, 2001; Imhof *et al.,* 2004; Pomp *et al.,* 2008). In most of these studies participants were Caucasian. Currently no information is available regarding the association between alcohol, fibrinogen concentration and PAI-1 in black Africans.

PAI-1 has been shown to be significantly lower and fibrinogen to be generally higher in black Africans when compared with levels typically observed in Caucasians (Jerling *et al.,* 1994; Vorster *et al.,* 1998; Festa *et al.,* 2003; Pieters *et al.,* 2006; Greyling *et al.,* 2007; Nienaber *et al.,* 2008). This raises the question of whether PAI-1 and fibrinogen levels will associate with habitual alcohol consumption in the same manner as they do in Caucasians, despite the observed differences in absolute levels.

The remainder of this literature review will deal with the following: the metabolism of alcohol; two biomarkers, gamma glutamyl transferase (GGT) and % carbohydrate deficient transferrin (%CDT), used to determine chronic alcohol consumption; and the effect of alcohol on health, including drinking patterns, negative effects of alcohol misuse and CVD health benefits related to moderate amounts of alcohol. An overview of coagulation and fibrinolysis is given and the role of fibrinogen and PAI-1 in CVD will be discussed. The effect of alcohol on PAI-1 and fibrinogen is tabulated for cross-sectional epidemiological, intervention (lasting 30 days to twelve weeks) and acute intervention (post-prandial type) studies, and an overview is given of drinking patterns of black South Africans as well as of the differences in PAI-1 and fibrinogen concentration between black Africans and Caucasians.

2.2. ALCOHOL METABOLISM

2.2.1. Absorption and metabolism of alcohol

Alcohols are a class of organic compounds containing hydroxyl (OH^-) groups (Figure 2.1). The particular kind of alcohol found in alcoholic beverages is called ethyl alcohol, or ethanol, which contains two carbons and one hydroxyl group (Whitney & Rolfes, 2005:240).

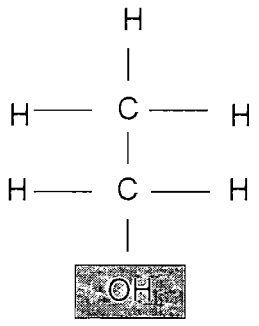

Figure 2.1. Chemical structure of ethanol

When ethanol is ingested in the form of an alcoholic beverage, approximately 20% is absorbed through the stomach wall, depending on the fed state of the individual consuming the beverage (Pawan, 1972; Whitney & Rolfes, 2005:241). In the stomach, alcohol dehydrogenase (gastric ADH) begins to break down ethanol. During this process, nicotinamide adenine dinucleotide (NAD^+) is reduced to NADH (Whitney & Rolfes, 2005:241; Clemens, 2006). The metabolism of ethanol in the stomach by gastric ADH is also known as first pass metabolism (Seitz, 1994). This enables the body to metabolise a fraction of the ingested ethanol before it reaches the duodenum, where the absorption area is much larger than the stomach (Norberg, 2003). After ethanol is absorbed through the duodenum, the alcohol-laden blood is transported to the liver (Whitney & Rolfes, 2005:242). In the liver cell, ethanol is primarily metabolised by three enzymatic pathways (Nagy, 2004) (see Figure 2.2).

The main pathway of oxidative metabolism of ethanol is located in the cytosol of the liver cell, and involves ADH (Caballería, 2003; Nagy, 2004; Caballería, 2005; Zakhari, 2006). One of the main products formed during metabolism of ethanol by ADH is acetaldehyde, which is a highly toxic by-product that may contribute to tissue damage and is also associated with unpleasant symptoms, including headache, nausea, an irregular rapid heartbeat and flushing (Paton, 2005; Zakhari, 2006). During this oxidation process, NAD^+ acts as an intermediate carrier of electrons and is reduced to NADH, which leaves

the liver cells vulnerable to damage from by-products of ethanol metabolism, such as free radicals and acetaldehyde (Zakhari, 2006).

Another pathway is the microsomal ethanol oxidising system (MEOS) in the endoplasmic reticulum of liver cells, which oxidises not only ethanol, but also several other classes of drugs (Caballería, 2003; Whitney & Rolfes, 2005:244). At high concentrations and in chronic alcoholism the importance of the MEOS for metabolism of ethanol is increased (Caballería, 2005). The metabolic tolerance to ethanol observed in alcoholics can be attributed to the induction of cytochrome P4502E1, which is a key enzyme of the MEOS which is up-regulated by chronic alcohol consumption (Lieber, 1997; Caballería, 2003; Zakhari, 2006).

The third enzymatic pathway in the liver is the catalase pathway in the peroxisomes, in which catalase acts as a catalyst to break down hydrogen peroxide to water and oxygen (Caballería, 2003). This system, however, plays a very small role in the metabolism of ethanol.

All these systems lead to the production of acetaldehyde (Caballería, 2003). Acetaldehyde dehydrogenase (ALDH) rapidly converts acetaldehyde to acetate and NADH (Zakhari, 2006). NADH is then oxidised by the mitochondrial electron transport chain to prevent accumulation and the subsequent inhibition of the tricarboxilic acid (TCA) cycle and lactic acid build-up (Whitney & Rolfes, 2005:243; Zakhari, 2006). Acetate is then converted to Acetyl CoA which enters the TCA cycle to generate energy (Whitney & Rolfes, 2005:242). When more acetate is formed than the TCA cycle can metabolise, the excess acetate is released into the circulation and will eventually be metabolised to CO2 in the heart, skeletal muscles and the brain (Zakhari, 2006).

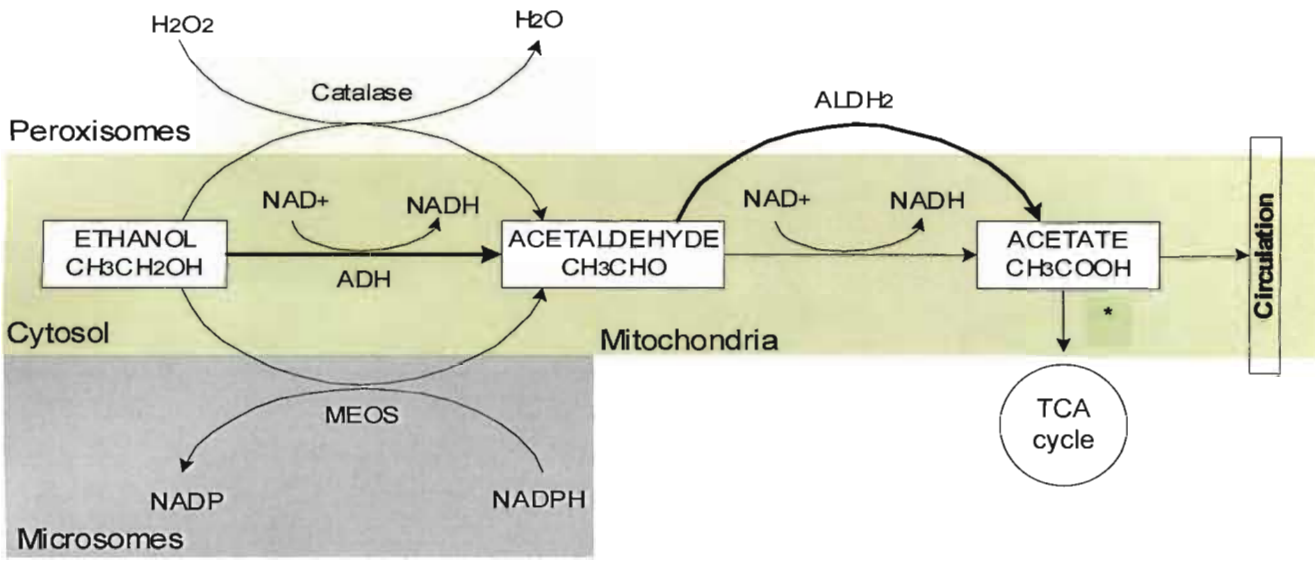

Figure 2.2. Oxidative metabolism of ethanol (Adapted from Zakhari, 2006 & Pisa *et al.*, in press)
ALDH: acetaldehyde dehydrogenase; NAD^+: nicotinamide adenine dinucleotide; NADH: reduced nicotinamide adenine dinucleotide; ADH: alcohol dehydrogenase; NADP: nicotinamide adenine dinucleotide phosphate; MEOS: microsomal ethanol oxidising system; NADPH: reduced nicotinamide adenine dinucleotide phosphate; TCA cycle: tricarboxilic acid cycle; *: rate limiting, when NADH accumulates, the TCA cycle is slowed down and this causes pyruvate and acetyl CoA to build up, and excess acetyl CoA is synthesised as fatty acids

The literature also describes non-oxidative pathways for metabolising ethanol (Figure 2.3). Deficient ADH activity due to liver damage facilitates the shift from oxidative ethanol metabolism to non-oxidative ethanol metabolism, which is predominantly found in chronic alcohol abuse (Vidal *et al.,* 1990; Wu *et al.,* 2006). Fatty acid ethyl esters (FAEEs) and phosphatidyl ethanol (PEt) are both products from the non-oxidative metabolism of ethanol (Wu *et al.,* 2006). Liver cells metabolise ethanol as an energy source, leaving fatty acids to accumulate and fatty acid ethyl esters (FAEEs) to form. With non-oxidative ethanol metabolism this production of FAEEs is increased (Whitney & Rolfes, 2005:242; Zakhari, 2006). The metabolism of ethanol can change liver cell structure, impairing the ability of the liver to metabolise fatty acids and causing the development of fatty liver disease (Whitney & Rolfes, 2005:242). The formation of the phospholipid PEt may result in disruption of cell signalling (Zakhari, 2006).

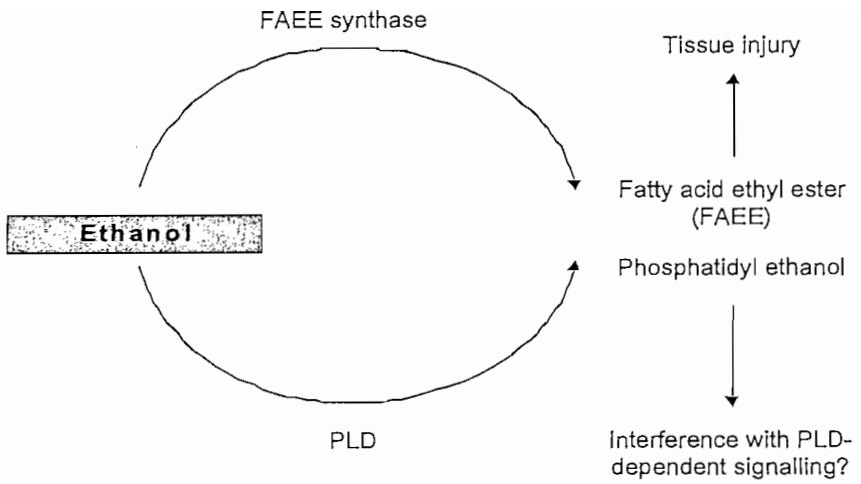

Figure 2.3. Non-oxidative metabolism of ethanol (From Zakhari, 2006).
FAEE synthase: Fatty acid ethyl ester synthase; PLD: phospholipase D

Various factors determine the rate of absorption of alcohol. High concentrations (20-30%) of ethanol in a drink will cause rapid absorption into the system, whereas a drink with an even higher ethanol concentration of 40%, like spirits, will cause delayed gastric emptying and delay absorption (Paton, 2005). Drinks like whisky mixed with soda, sparkling wines and champagne will also be absorbed quickly because of the carbon dioxide with which these drinks are carbonated (Paton, 2005). The amount of alcohol and the time span of drinking will also play a role in the rate of absorption as well as in the blood alcohol concentration (BAC) of the drinking individual, with higher amounts of alcohol ingested within short periods resulting in higher blood alcohol concentrations (Paton, 2005). One unit of alcohol, which constitutes a standard beverage containing 10-12 g of pure ethanol, will elevate the BAC of a man by 15 mg and of a woman by 20 mg per 100 ml within an hour of consumption (Sadler, 2007). Depending on body size, food intake, previous drinking experience and general health, the liver is able to process approximately 15 grams of ethanol per hour (Whitney & Rolfes, 2005:242). The ingestion of a meal before alcohol intake will slow the uptake of alcohol through the stomach wall and this will result in a lower maximum blood alcohol concentration (Norberg *et al.*, 2003; Paton, 2005).

Gender, body composition and size may also influence the metabolism of alcohol (Norberg *et al.*, 2003). Lower levels of ADH are present in women and thus the ability to metabolise ethanol before absorption into the circulation is hampered (Paton, 2005). Smaller body size, smaller blood volume and more subcutaneous fat in women also predisposes them to higher blood and tissue alcohol concentrations, as alcohol does not enter fat tissue, owing to poor solubility, therefore remaining in higher concentrations in the water parts of the body (Paton, 2005). Other factors which influence the rate of absorption and metabolism of alcohol are ethnic background and genetic factors such as differences between different populations in frequencies of the ADH1B*1 gene (which is predominant in Caucasian and black populations) of the ADH1B class of ADH (Zakhari, 2006). Two to five percent of ingested alcohol is excreted unchanged in urine, sweat and breath (Paton, 2005).

2.2.2. Biomarkers: %CDT and GGT

Owing to the unreliability of self-reported alcohol consumption data, especially in persons who use alcohol excessively, there is a need for clinical biomarkers to determine the actual amounts of alcohol consumed (Baros *et al.*, 2008). Two biomarkers which are currently being used to assess alcohol abuse are gamma-glutamyltransferase (GGT) and carbohydrate-deficient transferrin (CDT) (Niemelä, 2007).

CDT, the biological functions of which are still unknown, is primarily produced in the liver and is an isoform of transferrin (Mundt, 2004). Human transferrin can be found in at least six isoforms, namely penta-, tetra-, tri-, di-, mono-, and asialo-transferrin (Mundt *et al,.* 2004). The asialo, monosialo, and disialo isoforms of transferrin are considered to be carbohydrate-deficient and are therefore referred to as CDT isoforms. These are elevated in individuals who consume excessive amounts of alcohol and the CDT test is therefore used to detect alcohol abuse because of its high specificity (Mundt, 2004; Niemelä, 2007). CDT can be expressed in several ways. More recent results of CDT, which used to be expressed simply as units (U/l), are now given as a percentage of total transferrin (%CDT), thus taking into account varying concentrations of total transferrin in

different individuals (de Feo *et al.*, 1999; Anton *et al.*, 2001). Women in general seem to have higher CDT levels than men have, and the use of %CDT may improve the sensitivity of detection of CDT in women, particularly during pregnancy and hormone replacement conditions, during which CDT and transferrin levels are even higher (Stauber, 1996; Anton *et al.*, 2001). In conditions where altered transferrin production is present, as in chronic illness, liver disease and anaemia, the use of %CDT may also improve the sensitivity and specificity of the CDT test (Anton *et al.*, 2001).

It is possible, however, to obtain a false positive diagnosis of alcohol abuse using CDT. Bortolotti *et al.* (2006) summarised the clinical conditions leading to false positive diagnosis of alcohol abuse. The researchers included end-stage liver disease, catabolic diseases due to psychiatric disorders, anti-epileptic medicines, autoimmune hepatitis Type 1 and advanced liver disease. Furthermore, therapy with angiotensin II receptor blockers and total body water were conditions reported to lower concentrations of serum CDT, thus producing false negative results (Bortolotti *et al.*, 2006).

The other currently used biomarker of alcohol abuse is the membrane-bound glycoprotein liver enzyme, GGT, which acts as a donor substrate by binding glutathione (Keillor *et al.*, 2005). GGT is increased in serum when alcohol is consumed excessively, but the mechanism for this is still not clear (Bortolotti *et al.*, 2006; Niemelä, 2007). Various confounders may, however, also influence GGT test results. The sensitivity for GGT as a marker of excessive alcohol use has been shown to be lower for women than for men (Anton & Moak, 1994); in men, obesity can increase serum levels of GGT (Daeppen *et al.*, 1998) and GGT has, furthermore, been shown to increase with increasing age (Puuka *et al.*, 2006).

In a black African population, such as the South African Prospective Urban and Rural Epidemiological (PURE) study population, the accuracy of %CDT and GGT for the assessment of alcohol consumption or misuse is not entirely known (Pisa *et al.*, in press). The South African PURE study is part of the international PURE study which aims to track transition in sixteen countries around the world in various stages of transition. The

South African study population consists of 2010 participants. Approximately 1000 participants from rural areas and 1000 participants from urban areas, approximately equal numbers of men and women, aged between 35 to 65 years, make up the study population. For this reason a study was undertaken in which %CDT and GGT values of participants of the PURE study were compared with reported alcohol intake, using quantitative food frequency questionnaires (QFFQ) completed by the participants. The correlation between GGT and alcohol consumption was 0.43. Alcohol consumption and %CDT showed a correlation of 0.32 (Pisa *et al.,* in press). The researchers of the above-mentioned study thus concluded that these biomarkers were unsuitable for use in the African population in question, as using these biomarkers could lead to participants being incorrectly classified as drinkers (Pisa *et al.,* in press). It is also suggested that the current cut-off values for GGT and %CDT should be revised for use in an African population (Pisa *et al.,* in press). It is important to mention also that these biomarkers are traditionally used to determine alcohol abuse and may therefore not be applicable to all ranges of alcohol consumption. It is clear that, owing to the current problem of possible inaccurate reporting of alcohol consumption, there is still a significant demand for the development of biomarkers that accurately reflect alcohol consumption at all ranges of intake.

2.3. ALCOHOL AND HEALTH

2.3.1. Drinking patterns and amounts of alcohol consumed (guidelines)

Alcohol consumption guidelines produced by governments vary widely across different countries (ICAP, 2003). The amount considered to be a standard drink varies from a beverage consisting of 8 g of ethanol in the United Kingdom to 14 g in Portugal and the United States (ICAP, 2003). The South African National Council on Alcoholism and Drug dependence defines a standard drink (unit) as an alcoholic beverage containing 12 g of ethanol and advises men not to exceed 21 units per week and women not to consume more than 14 units per week (ICAP, 2003). Examples of standard drinks are 340 ml malt beer, 340 ml cider or cooler, a 25 ml tot glass of brandy, whiskey, gin, cane or vodka, or a

120 ml glass of wine, all of these drinks each containing approximately 12 g alcohol (Wolmarans *et al.,* 1992).

In South Africa the demographic and health surveys conducted in 1998 and 2003 documented Caucasian males and females as having the highest levels of alcohol intake, followed by Coloured and Indian males and females (DOH, 2007). Twelve and 14% of men and women respectively were found to be drinking at hazardous or harmful levels when reported drinking practices of the previous twelve months were analysed (DOH, 2007). Hazardous or harmful drinking over weekends with low or no intakes on weekdays (which is described as binge drinking) was found to be most prevalent in men aged 35-44 years and women of 65 years and older (DOH, 2007). Urban African men and Coloured men and women respectively are reported to have the highest level of harmful or hazardous drinking over weekends (DOH, 2007). Hazardous drinking over weekends is greatest among men from urban areas, whereas a higher proportion of women from non-urban areas practise alcohol consumption at hazardous levels (DOH, 2007).

2.3.2. Negative effects of alcohol misuse

The negative effects of alcohol misuse and abuse can be divided into three categories: the effect of alcohol on adult health, the effect on the family, and the negative effect in a social and economic context (van Heerden & Parry, 2001). The health hazards of alcohol abuse are summarised in Table 2.1.

Table 2.1. Hazardous effects and abnormalities in alcohol abuse (Adapted from James & Ralph, 2000)

Nervous system

Acute intoxication; 'blackouts'

Persistent brain damage: Wernicke's encephalopathy
Korsakoff's syndrome
Cerebellar degeneration
Dementia

Cerebrovascular disease

Strokes, especially in young people
Subarachnoid haemorrhage
Subdural haematoma after head injury

Withdrawal symptoms: Tremor, hallucinations, fits

Nerve and muscle damage: Weakness, paralysis, burning sensations in hands and feet

Liver

Infiltration of liver with fat; alcoholic hepatitis; cirrhosis and eventual liver failure; liver cancer

Gastrointestinal system

Reflux of acid into the oesophagus

Tearing and occasionally rupture of the oesophagus

Cancer of the mouth [b] and oesophagus

Gastritis

Aggravation and impaired healing of peptic ulcers

Diarrhoea and impaired absorption of food

Chronic inflammation of the pancreas leading in some to diabetes and malabsorption of food

Maldigestion and malabsorption of food due to complications associated with alcoholism (secondary malnutrition) [a]

Nutrition

Malnutrition from reduced intake of food and displacement of normal nutrients (primary malnutrition) [a], toxic effects of alcohol on intestine, and impaired metabolism (activation and utilisation of nutrients)[a] leading to weight loss.

Obesity, particularly in early stages of heavy drinking

Heart and circulatory system

Abnormal rhythms; high blood pressure; chronic heart muscle damage leading to heart failure

Respiratory system

Fractured ribs; pneumonia from inhalation of vomit

Endocrine system

Overproduction of cortisol leading to obesity, acne, increased facial hair and high blood pressure

Condition mimicking overactivity of the thyroid with loss of weight, anxiety, palpitations, sweating and tremor

Severe fall in blood sugar, sometimes leading to coma

Intense facial flushing in many diabetics taking the antidiabetic drug chlorpropamide

Reproductive system

In men, loss of libido, reduced potency, shrinkage in size of testes and penis, reduced or absent sperm formation and hence infertility, loss of sexual hair

In women, sexual difficulties, menstrual irregularities, and shrinkage of breasts and external genitalia

Coagulation system *

Increased fibrinogen levels in heavy drinkers

Increased alcohol consumption is associated with increased PAI-1

Other

Accidents with vehicles and auto-propelled machines [b]

Falls [b]

Self-inflicted harm and homicides [b]

[a] Lieber, 2000; [b] Meloni & Laranjeira, 2004; *Will be discussed in section 5

The misuse and abuse of alcohol can influence a child genetically and/ or prenatally (May *et al.,* 2007). Foetal alcohol syndrome (FAS), which is defined as a cluster of physical, behavioural and cognitive abnormalities associated with prenatal alcohol exposure, may occur when alcohol is consumed by pregnant women (Whitney & Rolfes, 2005:540). Alcohol drunk by a pregnant woman will cross the placenta to the foetus and blood alcohol levels will continue to rise until equilibrium is reached with the mother's blood alcohol levels (Whitney & Rolfes, 2005:540). Because of the undeveloped detoxification system and small size of the foetus, alcohol takes longer to leave the system and can cause detrimental damage (Whitney & Rolfes, 2005:540). In certain South African communities FAS incidence is the highest in the world and still rising (May *et al.*, 2007).

The negative social and economic effects of excessive alcohol use have far-reaching consequences (van Heerden & Parry, 2001). Social problems associated with alcohol include vandalism, public disarray, problems within the family such as marital conflict and divorce, interpersonal problems, child abuse, financial difficulties, educational difficulties, occupational problems other than health occupational problems, and social costs (Meloni & Laranjeira, 2004).

Moderate alcohol consumption, on the other hand, is considered to have possible CVD protective effects. This will be discussed in the following section.

2.3.3. CVD health benefits related to drinking moderate amounts of alcohol

The consumption of moderate quantities of alcohol appears to carry certain health benefits (Collins *et al.*, 2009). In fact, risk of mortality due to coronary heart disease (CHD), and amount of alcohol consumed can be plotted as a U- or J-shaped curve, with abstainers and heavy alcohol consumers carrying the highest risk and moderate consumers the lowest (Marmot & Brunner, 1991; Cleophas, 1999; Lucas *et al.*, 2005). Agarwal (2002) summarised the cardioprotective effects of moderate alcohol intake, which include increased HDL-cholesterol, inhibition of LDL-cholesterol, reduced platelet aggregation, reduced fibrinogen level, increased fibrinolysis, increased coronary blood flow, reduced blood pressure, reduced blood insulin levels, increased blood insulin

sensitivity, increased oestrogen levels, reduced stress and decreased plasma homocysteine levels. Controversy still exists, however, regarding whether it is the ethanol or other components, such as polyphenols in red wine, or whether it is both that are responsible for the beneficial effect seen with moderate alcohol consumption.

This dissertation will focus specifically on the effect of alcohol consumption on the haemostatic risk factors PAI-1 and fibrinogen.

2.4. HAEMOSTATIC FACTORS IN CVD

2.4.1. Overview of coagulation and fibrinolysis

2.4.1.1. Coagulation

The human haemostatic system is intricately designed to maintain the fluid state of blood under physiological conditions, but also to inhibit blood loss by sealing the damaged vessel wall in case of vascular injury (Colman, 2000:3). Under normal physiological conditions, one of the functions of the vascular endothelium is to provide a protective barrier between blood and plasma factors and the reactive elements within the deep layers of the vessel wall (Colman, 2000:3). The process of coagulation is viewed as a cascade of reactions which are divided into three overlapping steps, namely the initiation, amplification and propagation phases (Hoffman & Monroe, 2001; Frédérick *et al.*, 2005) (See Figure 2.4).

Initiation phase

In the event of damage to the vessel wall, plasma comes into contact with tissue factor (TF) from outside the blood vessel lumen, which then binds to plasma factor VII (Ajjan & Grant, 2006). Activated FVII/TF complexes activate plasma factor IX and factor X and these in turn activate factor V (Ajjan & Grant, 2006). The activated factor X and factor V will now generate a limited amount of thrombin by cleaving prothrombin, thus activating platelets (Ajjan & Grant, 2006). Furthermore, collagen fibres present in the vessel wall become exposed to blood and the activated platelets adhere to these collagen fibres (Briede *et al.*, 2001) (see figure 2.4).

Amplification phase

The collagen-bound platelets are only partly activated and the addition of more thrombin will enhance levels of pro-coagulant activity by fully activating platelets as well as factors V, VIII and XI (Frédérick *et al.*, 2005; Monroe & Hoffman, 2006). Von Willebrand factor (vWF) is further responsible for the adhesion of platelets to the damaged endothelium and binds factor VIII by forming non-covalent complexes (Ajjan & Grant, 2006). This vWF/factor VIII complex binds to platelets and factor VIII is cleaved from vWF by thrombin in order for factor VIII to be activated and more thrombin to be generated (Hoffman & Monroe, 2001; Frederick *et al.*, 2005).

Propagation phase

In the propagation phase, fully activated platelets change shape in order to enable factor IXa to bind to factor VIIIa on the platelet surface, additional factor IXa to be supplied by platelet-bound factor XIa, provision of factor Xa from the factor IXa/VIIIa complex to platelet surface to take place, and rapid association of factor Xa with the platelet surface factor Va to occur in order to produce enough thrombin to clot fibrinogen (Monroe & Hoffman, 2006).

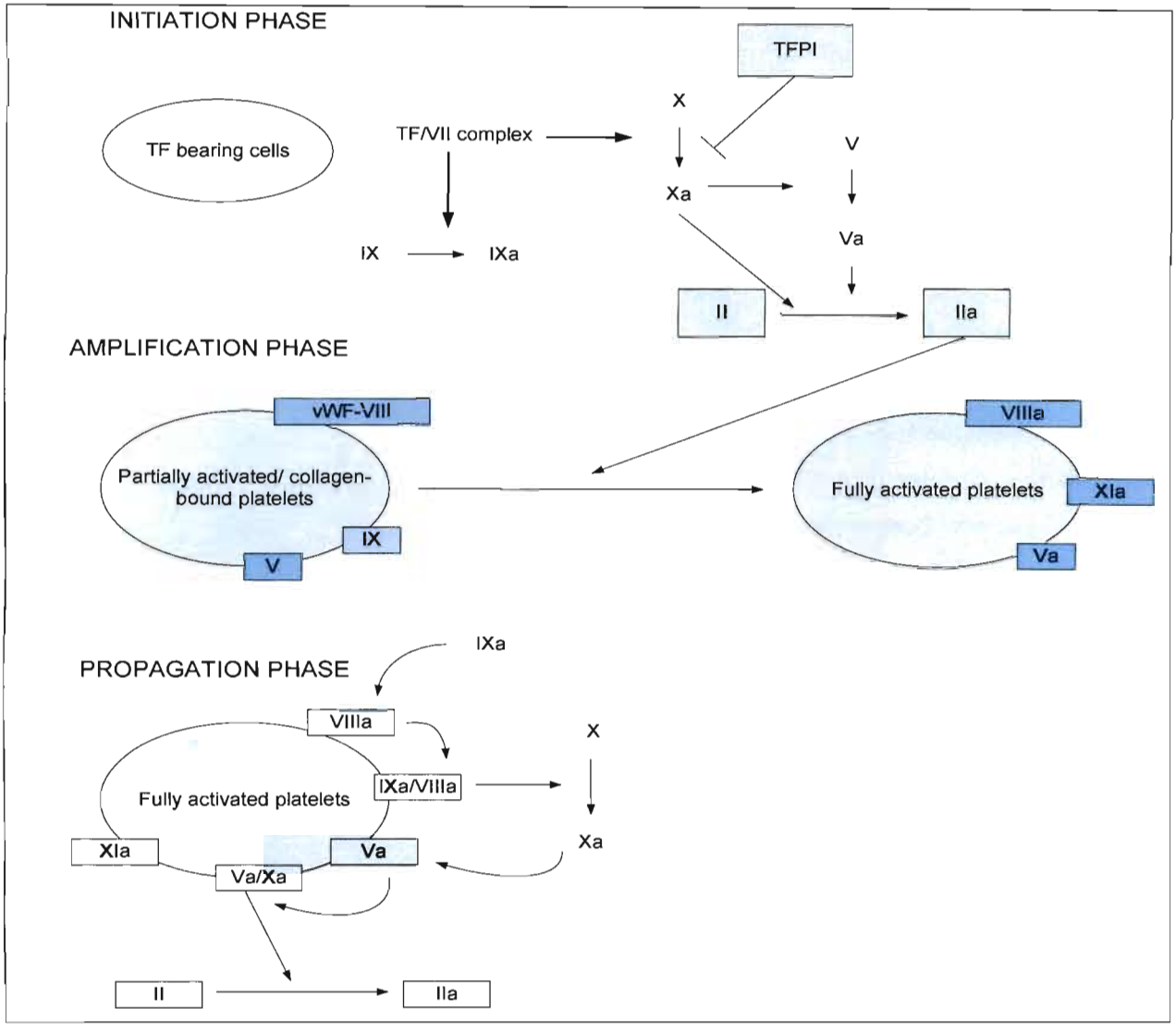

Figure 2.4. The coagulation cascade depicting the initiation, amplification, and propagation phases (adapted from Ajjan & Grant, 2006). TF: Tissue factor; TFPI: Tissue factor pathway inhibitor; vWF: von Willebrand factor.

Fibrinogen is a 340-kD glycoprotein which is made up of two symmetrical sets of three polypeptide chains (Aα, Bβ and γ) which are held together with disulfide bonds (Ajjan & Grant, 2006). It is normally present in plasma in concentrations of 2-3 g/l and has a half life of three to four days (Cook & Ubben, 1990; Baron, 2004; Standeven *et al.*, 2005). Thrombin produced in the propagation phase of haemostasis binds to fibrinogen to cleave activation peptides A and B from the Aα- and Bβ-chains to form fibrin monomers (Medved *et al.*, 2001). This enables interaction between adjacent fibrin monomers and

thus a fibrin clot is formed (Colman, 2000:11). The clot is further stabilised by thrombin and calcium which activates factor XIII to cross-link the fibrin fibres by a transglutaminase reaction (Ariëns *et al.,* 2002).

The structure of fibrin clots is influenced by several factors such as genetic polymorphisms, environmental influences, splice variations, disease in the human body and concentrations of kinetic and modulating factors present in plasma, such as fibrinogen, thrombin, calcium, albumin and fibronectin (Blombäck *et al.,* 1992; Standeven *et al.*, 2005). The level of fibrinogen is a major determinant of clot structure, and can itself be influenced by environmental, genetic and disease factors, including increasing age, female gender, smoking, obesity, physical inactivity, increased cholesterol levels, menopause, oral contraception use, low socio-economic status and stress, which all lead to increased plasma fibrinogen levels (Kamath & Lip, 2003; Standeven *et al.*, 2005).

Various mechanisms exist for the control and localization of haemostasis (Colman, 2000:12). These include the disruptive effect of blood flowing through a vessel and carrying small clumps of inadequately attached platelets away from the clot; the inhibitory effect of thrombomodulin on thrombin already present in the clot; soluble activated coagulant proteins which diffuse away from the clot instead of binding to it; and the diffusion of thrombin into the endothelial cell surface and restraint on local coagulation, which are a few of the mechanisms which stop the formation of clots (Colman, 2000:12). Existing clots can, however, also be broken down by a process called fibrinolysis, which is the major mechanism of clot dissolution (Figure 2.5).

2.4.1.2. Fibrinolysis

Tissue plasminogen activator (t-PA) is synthesised in endothelial cells. Two thirds of t-PA are, however, found in complex with its inhibitor PAI-1 (Thelwell & Longstaff, 2007). During lysis, free t-PA and plasminogen, a glycoprotein produced in the liver and containing lysine binding sites, bind to specific binding sites (lysine residues) on fibrin, cleaving it at these binding sites (Hoylaerts *et al.*, 1982; Tran-Thang *et al.*, 1984). Fibrin stimulates the activation of the zymogen plasminogen by t-PA to form active plasmin, and

also acts as a substrate for already formed plasmin (Hoylaerts *et al.*, 1982; Harpel *et al.*, 1985; Kohler & Grant, 2000). Fibrinolysis is accelerated, as early fibrin degradation by plasmin leads to new binding sites (lysine residues) being opened on the surface of fibrin for t-PA and plasminogen to bind to (Suenson *et al.*, 1984; Higgins & Vehar, 1987). The clot is subsequently degraded into small soluble fibrin fragments (Mosnier & Bouma, 2006).

Inhibition of clot breakdown may occur on plasmin or t-PA level through the action of several inhibitors (Collen & Lijnen, 1991). During coagulation small amounts of α_2-antiplasmin, which is the main inhibitor of plasmin, become cross-linked within the fibrin clot (Sakata & Aoki, 1982; Collen & Lijnen, 1991). These α_2-antiplasmin glycoproteins bind to fibrin-bound and free plasmin in order to inhibit fibrinolysis (Christiansen *et al.*, 2007). Other factors which inhibit fibrinolysis are the thrombin activatable fibrinolysis inhibitor (TAFI), which inhibits fibrinolysis by modulating the function of the fibrin cofactor for plasmin generation, and α2-macroglobulin, which "traps" plasmin and in this way inhibits the process of fibrinolysis (Pizzo & Wu, 2000; Mosnier & Bouma, 2006). PAI-1 is the main inhibitor of t-PA and a member of the serpin (serine protease inhibitor) family and, as mentioned earlier, two thirds of plasma t-PA are bound to PAI-1 (Diebold *et al.*, 2008). It is a single-chain glycoprotein with a molecular mass of about 50 KDa (Binder *et al.,* 2002). It occurs in plasma mainly bound to vitronectin, which increases its half life twofold to fourfold, and is removed from the circulation mainly by the liver (summarised by Hoekstra *et al.,* 2004). Currently it is suggested that the primary sources of PAI-1 may be the hepatocytes, adipocytes, platelets, endothelial cells and vascular smooth muscle cells (VSMC), but uncertainty still exists regarding which of these sources is the predominant origin of circulating PAI-1 under normal and different pathological conditions (Dellas & Loskutoff, 2005). PAI-1 inhibits fibrinolysis by binding and inhibiting t-PA (Diebold *et al.*, 2008). The binding of t-PA by PAI-1 results in clot stabilisation and prevention of premature lysis (Kohler & Grant, 2000). The possible negative effects of the inhibition of clot dissolution by PAI-1 will be discussed in the following section.

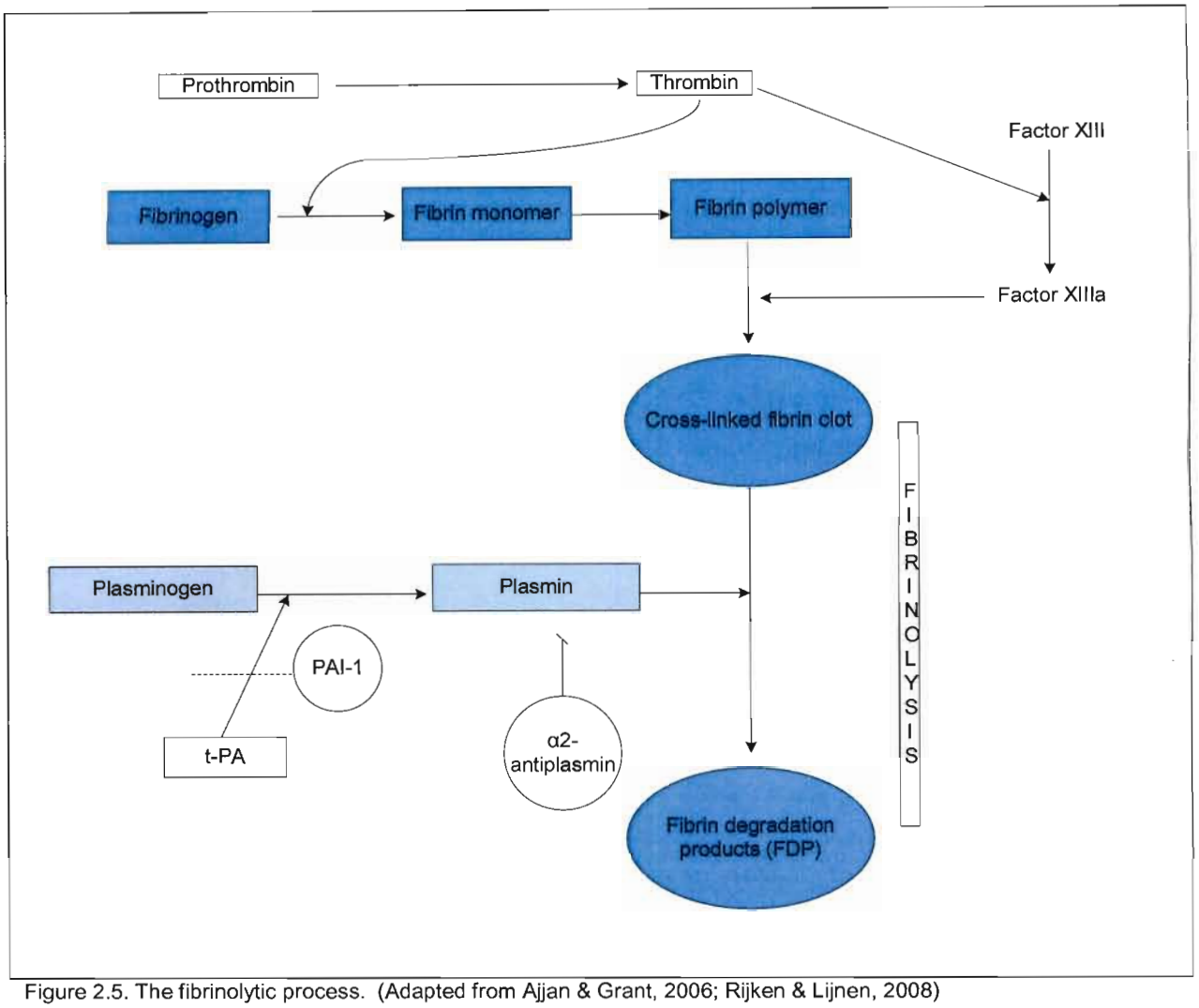

Figure 2.5. The fibrinolytic process. (Adapted from Ajjan & Grant, 2006; Rijken & Lijnen, 2008)

2.4.2. Plasminogen activator inhibitor type-1 in CVD

Owing to the inhibitory effect PAI-1 exerts on fibrinolysis, it has been suggested that it plays a causal role in CVD (Hawkins, 2004). PAI-1 is normally found in plasma at levels of 5-20 ng/ml (Binder *et al.*, 2002). Evidence exists that high plasma PAI-1 levels, which will lead to more t-PA being bound and enhanced suppression of fibrinolysis, are associated with the progression of coronary artery disease (CAD) and the development of myocardial infarction (MI) (Kohler & Grant, 2000; Hawkins, 2004). Various studies have found associations between low plasma fibrinolytic activity due to increased PAI-1

and CVD, but, in many of these studies the association disappears after adjustment for known risk factors such as smoking, a sedentary lifestyle, dyslipidaemia, high blood pressure, diabetes mellitus and being overweight (summarised by Folsom *et al.,* 2001). Elevated plasma PAI-1 concentrations were shown to be associated with MI in patients before the age of 45 years (Hamsten *et al.,* 1985), as well as with re-infarction (Hamsten *et al.,* 1987). Wiman *et al.* (2000) reported increased levels of t-PA/PAI-1 complex to be a risk marker for recurrent infarction in men and women, and Meade *et al.* (1993) found low fibrinolytic activity to be a leading determinant of ischaemic heart disease. PAI-1 seems to be an important risk marker in CVD.

Various factors determine plasma PAI-1 levels. Obesity, and especially central obesity, has been shown to be associated with higher PAI-1 levels (Ferguson *et al.*, 1998; Nienaber *et al.*, 2008) due to adipocytes being a source of PAI-1 (Dellas & Loskutoff, 2005). Other factors which are associated with increased PAI-1 levels have been summarised by Hoekstra *et al.* (2004) and include an unfavourable blood lipid profile, insulin resistance and diabetes, decreased oestrogen levels, and excessive alcohol consumption. PAI-1 levels are also influenced by genetic determinants.

Within the human PAI-1 gene, several polymorphisms have been described. The 4G/5G polymorphism in the promoter region of the PAI-1 gene is the most extensively studied with regard to PAI-1 levels (Figure 2.6) (Kohler & Grant, 2000; Hoekstra *et al.*, 2004). Subjects homozygous for the 4G allele generally present higher plasma PAI-1 concentrations than the 5G/5G or heterozygous genotype (Dawson *et al.*, 1991; Eriksson *et al.*, 1995; Panahloo *et al.,* 1995; Ye *et al.*, 1995). This polymorphism is described as a response polymorphism because differences in PAI-1 levels become more obvious in subjects homozygous for the 4G or 5G allele in the presence of environmental and/or disease factors leading to increased PAI-1 expression (Hoekstra *et al.*, 2004). The reason for this might be that, even though both alleles bind a transcriptional activator, the 5G allele also binds a repressor protein to an overlapping binding site, which decreases binding of the activator because of interference caused by steric hindrance (Sartori *et al.*, 2001).

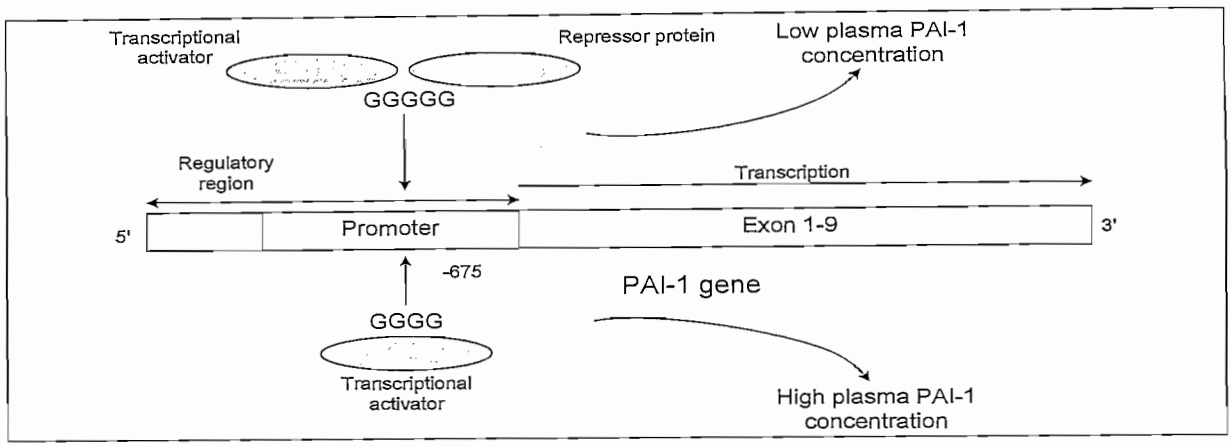

Figure 2.6. Site of the 4G/5G polymorphism in the promoter region of the PAI-1 gene (Kohler & Grant, 2000)

In plasma, PAI-1 can be measured as either PAI-1 activity ($PAI\text{-}1_{act}$) or as PAI-1 antigen ($PAI\text{-}1_{ag}$). All forms of PAI-1, namely active and inactive or latent, free or in complex with t-PA and/or vitronectin, are measured in the PAI-1 antigen assay, while the PAI-1 activity assay measures only active PAI-1 (the amount that can actively inhibit t-PA).

2.4.3. Fibrinogen in CVD

A few years ago fibrinogen was said to be strongly related to cardiovascular risk and was classified as a strong and independent risk factor for CVD (Mertens & van Gaal, 2002; Kamath & Lip, 2003; Koenig, 2003). However, more recently Donati and Iacoviello (2007) suggested that fibrinogen should be considered rather as an effective intermediate link between classical risk factors and CAD, instead of being regarded as an independent predictor of disease. A direct association has, however, been found between the fibrinogen level and severity of CAD (Jackson *et al.*, 2000). Fibrinogen levels have been shown to be elevated after acute thrombosis and in patients with unstable angina or infarction (Koenig, 2003). The reasons why elevated fibrinogen levels are seen in CVD and atherosclerosis in general are not completely understood (Koenig, 2003). Potential pathophysiological mechanisms which may link elevated fibrinogen levels to atherogenesis and its thrombotic complications include the role of fibrinogen in the

coagulation cascade as the substrate for thrombin, the essential role it plays in platelet aggregation, modulation of endothelial function, and the promotion of proliferation and migration of smooth muscle cells (Koenig, 2003).

2.5. EFFECT OF ALCOHOL ON FIBRINOGEN AND PAI-1

In Table 2.2, studies reporting the effect of ethanol on fibrinogen are summarised. The table is divided into cross-sectional epidemiological studies, intervention studies (lasting from 30 days to twelve weeks) and acute intervention (post-prandial type) studies. Table 2.3 consists of cross-sectional epidemiological studies, intervention studies lasting 30 days to twelve weeks, and acute intervention (post-prandial type) studies reporting on the effect of ethanol on PAI-1.

Table 2.2. Fibrinogen: Cross-sectional epidemiological studies

Study	Participants	Ranges of alcohol consumed	Type of alcohol consumed	Results Fibrinogen concentration	Conclusions
Meade *et al.*, 1979	1601 male and 707 female participants aged 18-64 and 18-59 years respectively	Participants divided into drinkers (those who consume alcohol on a weekly basis), and non-drinkers (teetotallers and those reporting alcohol intake at less than weekly intervals)	Mostly beer consumed	Only fibrinogen (g/l) of men reported: Drinkers (mean intake 22.8 ± 22.9 g/day): 2.75 Non-drinkers: 2.89	Plasma fibrinogen concentration was decreased with alcohol consumption.
Folsom *et al.*, 1991	Participants from the ARIC study aged 45-64 years (fibrinogen values for 11546 participants)	None, >0-100, >100g/week	Beer, wine and liquor	Fibrinogen (mg/dl): Men: None: 302; >0-100: 299; >100: 297 Women: none: 317; >0-100: 306; >100: 294	Fibrinogen negatively associated with alcohol intake.
Krobot *et al.*, 1992	434 (aged 25-74 years) participants of the MONICA Project Augsburg survey, 1989-1990	None; 0.1-19.9; 20-39.9; 40-59.9; 60-79.9• and ≥80 g/day (men only) •≥60 g/day for women	Not included in questionnaire	0 g/day; 0.1-19.9 g/day; 20-39 g/day; 40-59.9 g/day; •60-79.9 g/day; ≥80 g/day **Men** 25-44 years (mg/dl): 371; 347; 335; 327; 337; 349 45-74 years (mg/dl): 437; 409; 408; 399; 391; 404 **Women** 25-44 years (mg/dl): 370; 353; 350; 321; 316 45-74 years (mg/dl): 429; 419; 402; 384; 415	U-shaped correlation between alcohol consumption and fibrinogen. This shape not as prominent in men older than 45 years, absent in young women and decreased with heavy alcohol consumption in men (aged 25-44 years). Lowest mean fibrinogen concentration at 40-59.9 g/day.

Lee *et al.*, 1995	1537 Participants (750 women, 787 men) from the Edinburgh Artery study	Men: None, 1-7, 8-18, ≥19 units/week Women: None, 1-3, 4-7, ≥8 units/week 1 Unit = 1glass of wine, half pint beer or single measure of spirits	Wine, beer, spirits	Fibrinogen (g/l): Men: None: 2.65, 1-7: 2.48, 8-18: 2.55, ≥19: 2.56 Women: None: 2.88, 1-3: 2.64, 4-7: 2.58, ≥8: 2.65	Among men and women alcohol intake was significantly and negatively associated with fibrinogen.
Marques-Vidal *et al.*, 1995	615 participants from France and Northern Ireland (aged 25-64 years) ECTIM study	0≤15; 15<·≤36; 36<·≤66 and >66 g/day. Non-drinkers comprised the fifth group	Wine, beer, cider and spirits	Fibrinogen (mg/dl): non-drinkers: 301 ± 8; 0≤15: 302 ± 7; 15<·≤36: 308 ± 8; 36<·≤66: 295 ± 7; >66 g/day: 293 ± 8	In this study the effect of alcohol on fibrinogen seems small. The group of participants who consumed <66 g/day had higher fibrinogen levels than the participants who consumed >66 g/day.
Mennen *et al.*, 1999a	4967 (2499 women, 2468 men) participants from DESIR study, mean age for men and women 47 ± 10 years	Non-drinkers; 0-19; 20-39; 40-59; 60-79 and ≥80 g/day	Wine, beer, cider and spirits	Men: 2.92 ± 0.64 g/l, women 3.08 ± 0.66 g/l. fibrinogen levels highest in non-drinkers and lowest in range 20-59g alcohol/day	U-shaped association between fibrinogen and alcohol consumption. Fibrinogen lowest in participants consuming 20-59 g/d, increased consumption led to higher fibrinogen concentrations.
Yarnell *et al.*, 2000	2223 male participants of the Caerphilly Study	ml of pure alcohol/week: <11; 11-48; 49-136; 137-229; ≥230	Not mentioned	Nephelometric fibrinogen concentration (g/l): <11: 3.90; 11-48: 3.97; 49-136: 3.92; 137-229: 3.86; ≥230: 3.73 Clottable fibrinogen concentration (g/l): <11: 3.03; 11-48: 3.11; 49-136: 3.01; 137-229: 2.93; ≥230: 2.83	Alcohol consumption seems to be negatively associated with both nephelometric and clottable fibrinogen. Participants consuming less than 48ml pure alcohol/ week had the highest fibrinogen concentrations.
Marques-Vidal *et al.*, 2001	6729 men aged 50-59 years at collaborating centres of the PRIME study (5362 France, 1367 Northern Ireland)	Analysis restricted to drinkers. Total alcohol consumption: France: 318 ± 249 ml ethanol/wk. Ireland: 325 ± 333 ml ethanol/wk French participants: 0-1890 ml wine/wk; 0-1750 ml beer/wk and 0-1701 ml spirits/wk. Participants from Northern Ireland: 0-661 ml wine/wk; 0-3584 ml beer/wk and 0-2272 ml spirits/wk	Wine, beer and spirits	France: Total alcohol consumption significantly and negatively associated with fibrinogen. Similar results found when individual beverage types where analysed: except NS correlation with beer consumption. Northern Ireland: Total alcohol consumption: no significant correlation with fibrinogen. Wine significantly and negatively correlated with fibrinogen, and beer significantly and positively correlated with fibrinogen.	Total alcohol consumption is related to fibrinogen, but the magnitude and nature of this effect depends on the type of alcoholic beverage.

Mukamal *et al.*, 2001	3223 (1456 men and 1767 women) participants from Framingham Offspring study without CVD	Non-drinkers; <3; 3 to <7; 7 to <21 and ≥21 drinks/week	Beer, wine and liquor	Women (mg/dl): 0 drinks/week: 324 ± 61.5; 1-<3 drinks/week: 309 ± 53.9; 3-<7 drinks/week: 302 ± 51.7; 7-<21 drinks/week: 290 ± 58.5. Men (mg/dl): 0 drinks/week: 312 ± 60.4; 1-<3 drinks/week:303 ± 54.5; 3-<7 drinks/week: 294 ± 52.9; 7-<21 drinks/week: 298 ± 59.9; ≥21 drinks/week: 301 ± 62.2	Moderate alcohol use was associated with lower levels of fibrinogen, lowest levels at 7-21 drinks/week. At higher levels of alcohol consumption fibrinogen appeared to reach a plateau or reverse. Similar results were found for all beverages.
Wannamethee *et al.*, 2003	The British Regional Heart Study. 3157 men, aged 60-79 years. No history of MI, stroke or diabetes	None, <1/day, 1-2/day, 3-4/day, ≥5/day (1 unit = 12-15g alcohol)	Beer, wine and spirits	Clottable fibrinogen (g/l) of various groups: no alcohol:3.30; <1 drink/day; 3.26; 1-2 dinks/day: 3.22; 3-4 drinks/day: 3.19; ≥5 drinks/day: 3.08	Inverse relationship between total alcohol consumption and fibrinogen. This effect most consistent in wine.
Mukamal *et al.*, 2004	4265 men and women free of clinical CVD, aged ≥65 years who participated in the CHS	None; former drinker; <1 drink/wk; 1-<7 drinks/wk; 7-<14 drinks/wk and >14 drinks/wk	Beer, wine and liquor	Fully adjusted fibrinogen levels of total group in mg/dl: None: 318 ± 1.5; former drinkers: 317 ± 3.3; <1 drink/wk: 320 ± 2.1; 1-<7 drinks/wk:311 ± 2.2; 7-<14 drinks/wk: 308 ± 3.6 and >14 drinks/wk: 290 ± 3.0	Alcohol consumption was found to be inversely and significantly related with fibrinogen.
Imhof *et al.*, 2004	5778 participants from the Augsburg and Glasgow MONICA 1994/95 groups aged 25-74 years. Both groups consumed mostly beer	None; >0-20; >20-40; >40-60; >60-80; >80 gram alcohol per day	Beer, wine and spirits	Total group fibrinogen (g/l): None: 3.01 ± 0.74; >0-20: 2.80 ± 0.67; >20-40: 2.69 ± 0.62; >40-60: 2.77 ± 0.66; >60-80: 2.78 ± 0.61; >80: 2.88 ± 0.75 Augsburg fibrinogen (g/l): None: 3.03 (2.99-3.08); >0-20: 2.89 (2.86-2.92); >20-40: 2.77 (2.72-2.82); >40-60: 2.76 (2.67-2.86); >60-80: 2.75 (2.60-2.89); >80: 2.74 (2.51-2.97) Glasgow fibrinogen (g/l): None: 2.74 (2.67-2.82); >0-20: 2.61 (2.57-2.65); >20-40: 2.60 (2.52-2.68); >40-60: 2.70 (2.53-2.87); >60-80: 2.63 (2.38-2.89); >80: 2.88 (2.45-3.30)	Moderate daily consumption of up to 40 g alcohol was associated with decreased fibrinogen concentrations when compared with non-drinking and heavy drinking groups.
Schröder *et al.*, 2005	4502 participants (men: 2201, women: 2301) aged 25-74 years.	0; 1-20; 21-30; >30 g/day	Wine, beer and spirits	An inverse relationship between alcohol and fibrinogen was seen in men and women.	In the study increasing alcohol consumption was associated with lower fibrinogen levels.

Pomp *et al.*, 2008	Patients, n = 4423 (mean age 48.5 years) (2400 women). Control group (mean age 46.8 years) consisting of random controls and patient partners (n=5235, n=2816 women)	None; ≤1glass/week; 2-6 glasses/week; 1 glass/day; 2-4 glasses/day; 5-9 glasses/day; 10-19 glasses/day; 20-29 glasses/day; 30-39 glasses/day and ≥40 glasses/day	Not included in question-naire	Mean fibrinogen levels of control subjects: No and ≤1 glasses of alcoholic beverages/wk: 3.40-3.50 g/l. 2-6/wk, 1/day, 2-4/day and 5-9/day: means vary around approximately 3.25 g/l. ≥10 glasses/day approximately 3.60 g/l	Authors concluded 2-4 glasses/day to be associated with reduced risk of venous thrombosis. Reduction may be attributed largely to lower fibrinogen levels.
Perissinotto *et al.*, 2009	1896 Italian men aged 65-84 years participating in the ILSA study	Lifelong abstainers; ≤12 g/day; 13-24 g/day; 25-47 g/day; 48-96 g/day, and >96 g/day	Wine, beer and spirits	Fibrinogen concentration (mg/dl): Abstainers: 352 ± 8.5; ≤12 g/day: 351 ± 5.5; 13-24 g/day: 338 ± 4.7; 25-47 g/day: 332 ± 5.6; 48-96 g/day: 330 ± 7.2 and >96 g/day: 327 ± 16.3	In elderly men there was a significant decrease of fibrinogen concentrations as alcohol consumption increased.
Tolstrup *et al.*, 2009	9584 participants from the Copenhagen City Heart Study, median age 61 years	Women: <1, 1-6, 7-13, 14-20, 21-27, 28+ drinks/week Men: <1, 1-6, 7-13, 14-20, 21-27, 28-35, >35 drinks/week	Beer, wine and spirits	Fibrinogen concentration (g/l): Women: <1: 2.72; 1-6: 2.69; 7-13: 2.55; 14-20; 2.45; 21-27: 2.44; 28+: 2.39 Men: <1: 2.64; 1-6: 2.60; 7-13: 2.55; 14-20; 2.48; 21-27: 2.46; 28-35: 2.45; 35+: 2.46	Among men and women alcohol consumption was statistically significantly associated with decreased fibrinogen concentrations.

CVD: cardiovascular disease ; CHS: Cardiovascular Health study ; MI: Myocardial infarction ; NS: not significant

Results from most epidemiological studies show a negative correlation for fibrinogen levels and alcohol intake (Meade *et al.*, 1979; Folsom *et al.*, 1991; Lee *et al.*, 1995; Marques-Vidal *et al.*, 1998; Yarnell *et al.*, 2000; Wannamethee *et al.*, 2003; Mukamal *et al.*, 2004; Shroder *et al.*, 2005; Perissinotto *et al.*, 2009; Tolstrup *et al.*, 2009). Some studies, however, found that fibrinogen concentrations reach a plateau at higher levels of alcohol consumption or even increase (Krobot *et al.*, 1992; Mennen *et al.*, 1999a; Mukamal *et al.*, 2001; Imhof *et al.*, 2004; Pomp *et al.*, 2008). In most studies the lowest fibrinogen levels are seen in participants consuming moderate amounts (1-4 glasses) of alcoholic beverages per day (Meade *et al.*, 1979; Krobot *et al.*, 1992; Lee *et al.*, 1995; Mennen *et al.*, 1999a; Mukamal *et al.*, 2001; Mukamal *et al.*, 2004; Imhof *et al.*, 2004; Pomp *et al.*, 2008; Perissinotto *et al.*, 2009). Of all alcoholic beverages investigated, wine seems to be the most consistent in this inverse association between fibrinogen and alcohol consumption (Marques-Vidal *et al.*, 2001; Wannamethee *et al.*, 2003).

Fibrinogen: Intervention studies (lasting 30 days to twelve weeks)

Study	Participants	Ranges of alcohol consumed	Type of alcohol consumed	Results	Conclusions
Pellegrini *et al.*, 1996	11 Healthy men aged 20-45 years	Six-month intervention during which 320ml red wine (containing 30 g alcohol), 320 ml clear fruit juice with 30 g alcohol or dealcoholised red wine was consumed daily with two main meals for three periods of four weeks. Each treatment period was preceded by four weeks of alcohol abstinence	Red wine, fruit juice with added alcohol and dealcoholised red wine	Fibrinogen concentration (mg/dl): Abstinence: 245 ± 10; Dealcoholised red wine: 255 ± 8; Alcohol (fruit juice drink): 229 ±6; Red wine: 240 ±13	Fibrinogen was significantly decreased with alcohol consumption, whereas consumption of dealcoholised red wine led to a small and non-significant ($p<0.08$) rise in fibrinogen.
Gorinstein *et al.*, 1997	28 males 51-73 years. Randomly divided between experimental (n=22) and control group (n=6)	Experimental group: 330 ml Maccabee beer (contains ± 20 g/alcohol) every day for 30 consecutive days. Control group did not consume any alcohol during this 30-day period	Maccabee beer	Fibrinogen levels of experimental group: baseline: 11.0-17.3 mmol/L. Control group: 11.0-17.3 mmol/L. After 30 days: experimental group: 10.4-17.5 mmo/L; control group: 11.0-17.3 mmol/L	No statistically significant changes were seen in fibrinogen levels within or between groups.
Dimmitt *et al.*, 1998	55 male participants aged 20-63 years, overweight (mean BMI= 26.8) Usual intake = 210-500ml absolute alcohol equivalent/ week	12-week study, four weeks baseline familiarisation period, following four weeks during which subjects were randomised either to continue their usual alcohol intake or to reduce their alcohol intake by consuming a low-alcohol beer	Swan beer (4.9%vol), Swan Special Light (0.9%vol)	Fibrinogen during low alcohol intake: 3.06 g/l, usual alcohol intake: 2.71 g/l	A significant reduction was seen in fibrinogen concentration with consumption of four standard drinks per day.
Minami *et al.*, 2002	33 Japanese male participants (mean age 36.9 ± 1.1 years)	Subjects habitually consumed 63.6 ± 6.3 ml ethanol daily. In this randomised cross-over design 17 participants kept to their usual drinking for a period of three weeks, thereafter a three-week period followed in which participants reduced their alcohol intake by at least half of habitual amounts. The remaining participants followed the same protocol but in the reverse order	Beer, sake, wine, whisky and other spirits	Plasma fibrinogen concentration (g/l): usual alcohol consumption: 2.34 ± 0.12; reduced consumption period: 2.30 ± 0.12	Fibrinogen levels did not significantly differ between these two periods.
Sierksma *et al.*, 2002	Ten men (45-64 years) and nine postmenopausal women (aged 49-62 years) in a randomised cross-over trial lasting six weeks	Five men and five women consumed four and three glasses of Amstel beer respectively with dinner every day for three weeks. Thereafter non-alcoholic beer of the same amounts was consumed for three weeks. The remaining nine subjects consumed the no-alcohol beer first, followed by three weeks consuming the beer	Amstel Beer, 5 vol% alcohol. Amstel Malt Beer, <0.1 vol% alcohol	Total group fibrinogen (g/l) for the non-alcoholic beer was 3.68 ± 0.68 and for the beer 3.22 ± 0.47. Men: non-alcoholic beer: 3.38 ± 0.58 g/l; beer: 3.05 ± 0.41 g/l. Women: non-alcoholic beer: 4.02 ± 0.64 g/l; beer: 3.42 ± 0.48 g/l.	After beer consumption, plasma fibrinogen levels were significantly decreased (12.4%). Beer consumption, compared with non-alcoholic beer led to significant reductions in plasma fibrinogen levels of both men and women.

Estruch *et al.*, 2004	40 healthy men (mean age 37.6 ± 7.4) participated in this randomised, cross-over, single-blinded trial lasting 86 days	During the first washout period which lasted 15 days participants did not consume any alcohol; during the following 28 days 20 participants drank 160 ml red wine and 20 drank 100 ml gin (beverages equal to 30 g alcohol/ day). A second washout period of 15 days followed, and thereafter 28 days during which participants who had received wine in the previous intervention drank gin and *vice versa*	12.5 vol% wine, gin	Fibrinogen (g/dl) for gin: Before: 0.27 ± 0.07, after: 0.24 ± 0.06. Red wine: Before: 0.26 ± 0.06, after: 0.23 ± 0.06	Plasma fibrinogen decreased significantly after 28 days of consuming 160 ml red wine or 100 ml gin.
Mezzano, 2004	Two groups of healthy males (aged 22 ± 3.4 years)	Intervention consisted of 90 days during which one group followed the Mediterranean diet (MD) for the first 30 days, the MD supplemented with 240 ml/day of red wine, and the last 30 days again following only the MD. The second group followed the same protocol, but consuming high fat (HF) diets	240 ml red wine every day for 30 days	Red wine consumption resulted in diet-independent decreases in fibrinogen. The fibrinogen concentrations of participants in the MD group decreased from ± 238 mg/dl to ± 223 mg/dl. The fibrinogen levels of participants consuming the HF diet decreased from ± 267 mg/dl to 226 mg/dl	Moderate amounts of red wine consumed with a MD or HF diet improved the haemostatic profile of individuals consuming this type of diet.
Hansen *et al.*, 2005	69 participants (men n=31, women n=38) aged 48-55 years. Participants were randomly assigned to four groups.	Study lasted four weeks, wine, tablets and placebos were consumed every day. Groups: red wine (men: 300 ml (38.3 g)); women: 200 ml (25.5 g); water and red grape extract (wine-equivalent dose of non-ethanol ingredients); water and red grape extract (½ of wine-equivalent dose of non-ethanol ingredients; water and placebo tablets	Domaine de Malepére, 1999 red wine based on Merlot and Cabernet-Sauvignon, containing 12.75% alcohol	Baseline values for fibrinogen in g/l: red wine: 3.23 (0.10); full dose extract: 3.11 (0.14); half dose extract: 2.96 (0.08); placebo: 3.09 (0.12). Changes after four weeks: red wine: 0.15 (0.07); full dose extract: 0.30 (0.17); half dose extract: 0.09 (0.06); placebo: 0.10 (0.08).	Compared with water with or without red fermented grape extract, red wine consumed in moderate amounts decreased fibrinogen levels by 8-15%.
Avellone *et al.*, 2006	48 apparently healthy participants aged 35-65 years (men=28, women=20)	Participants were assigned to two groups. Group A (n=24) received 250 ml red wine with meals for four weeks, after which they returned to their usual (<250 ml/ week) wine intake. Group B followed the same protocol but in the reverse order	12 participants of each group received Nero d'Avola and 12 Etna Torrepalino	Group A fibrinogen (mg/dl) Baseline: 336.2 ± 71.3; +4 weeks: 254.4 ± 49.6; +8 weeks: 338.5 ± 65.9. Group B fibrinogen (mg/dl): Baseline: 340.1 ± 72.1; +4 weeks:340.2 ± 70.7; +8 weeks:259.5 ± 48.5	Supplementation of the diet with red wine induced in group A at four weeks, and in group B at eight weeks (after four weeks of consumption) significant reductions in fibrinogen levels.

BMI: Body mass index

Intervention studies have been conducted ranging from three to twelve weeks, using 100 ml to 500 ml of alcoholic beverages in various forms (Pellegrini *et al.*, 1996; Gorinstein *et al.*, 1997; Sierksma *et al.*, 2002; Estruch *et al.*, 2004; Mezzano, 2004; Hansen *et al.*, 2005; Avellone *et al.*, 2006). Significant reductions in fibrinogen levels were found, especially in studies where red wine was the beverage used

(Mezzano, 2004; Hansen *et al.*, 2005 & Avellone *et al.*, 2006). Studies in which beer was used are less consistent, with Sierksma *et al.* (2002) and Dimmitt *et al.* (1998) finding significant reductions in fibrinogen levels of men and women as opposed to Gorinstein *et al.* (1997) who did not find significant changes in fibrinogen levels. In the latter study, 330 ml Maccabee beer (approximately 20 g alcohol) were given to men aged 51 to 73 years to ingest daily for 30 days, whereas the study by Sierksma *et al.* (1997) provided three (30 g alcohol) and four (40 g alcohol) glasses of Amstel beer to women and men respectively for three weeks, the ages of these participants being 49 to 62 years. Thus it is seen that in the study of Sierksma *et al.* (2002) a larger amount of pure alcohol was consumed over a shorter period of time resulting in decreased fibrinogen concentrations. Overall, 200 to 480 ml/day of most alcoholic beverages seem to reduce fibrinogen levels in men and women. The fact that fibrinogen concentrations were decreased in studies where red wine was consumed, as well as in some studies where beer (Dimmitt *et al.*,1998; Sierksma *et al.,* 2002) and gin (Estruch *et al.*, 2004) were consumed, together with the fact that studies using dealcoholised red wine (Pellegrini *et al.*, 1996) and tablets containing red grape extract (Hansen *et al.*, 2005) did not find decreased fibrinogen concentrations, may be an indication that it is the ethanol component of alcoholic beverages, not polyphenolic components in red wine, that is the reason for the decreased fibrinogen concentrations.

Fibrinogen: Acute intervention studies (post-prandial type)

Study	Participants	Ranges of alcohol consumed	Type of alcohol consumed	Results	Conclusions
Elmér *et al.*, 1984	Ten healthy volunteers (seven men and three women)	2 ml whisky/kg body weight was ingested after an overnight fast. Participants consumed the drink in a time span of 15 min. At 1 and 2 h after ingestion blood was collected	Whisky	No changes were seen in fibrinogen concentrations when compared with baseline concentrations.	Consumption of 2 ml whisky/kg body weight did not affect fibrinogen concentrations.
El-Sayed *et al.*, 1999	11 healthy, moderately trained participants (mean age 22.8 ± 1.4 years)	Participants each performed two exercise trials which were separated by seven days. 45 minutes before the first trial participants consumed 0.5 g/kg vodka diluted 1 in 4 with a glucose-free orange flavoured solution. On the second trial an equal volume of non-alcohol glucose-free orange-flavoured solution was ingested. Participants then cycled continuously at 65% VO2max for 30min, followed by an all-out self-paced performance of 5 min. Blood samples were collected 45 minutes after ingestion of beverage and immediately after exercise	Vodka	A significant reduction was observed for plasma fibrinogen concentration when post-exercise values were adjusted for plasma volume loss. This was seen in the alcohol, but not in the control trial.	Moderate consumption of alcohol may decrease plasma fibrinogen concentrations after exercise.

Tousoulis *et al.*, 2008	83 healthy young participants assigned to five groups in which equal amounts of alcohol were consumed.	Each beverage contained 30 g of alcohol. 264 ml red wine, 264 ml white wine, 633 ml beer or 79 ml whisky; the last group ingested 250 ml water. Blood samples were collected at baseline and 4h after consumption	Red and white wine, beer and whisky	Fibrinogen concentrations (mg/dl): Red wine - baseline: 250 ± 12, 4h: 235 ± 9; white wine - baseline: 219 ± 11, 4h: 217 ± 11; beer – baseline: 226 ± 26, 4h: 218 ± 27; whisky – baseline: 229 ± 15, 4h: 244 ± 17; water – baseline: 219 ± 10, 4h: 224 ± 10	Consumption of moderate amounts of red wine may decrease fibrinogen concentrations.

Acute intervention studies regarding alcohol consumption and its effect on fibrinogen concentration are scarce and results are inconclusive. The study by Tousoulis *et al.* (2008) found decreased fibrinogen levels after moderate consumption of red wine. Elmér *et al.* (1984) did not find that whisky affected fibrinogen levels at all. El-Sayed *et al.* (1999) conducted their research on participants before and after exercise, and for this reason the results cannot be compared with those of the other studies. Thus, owing to the limited number of studies conducted and the inconclusive results of these studies, it is not possible to draw a conclusion regarding the effect of acute alcohol consumption on fibrinogen concentration.

Table 2.3. Plasminogen activator inhibitor-type 1: Cross-sectional epidemiological studies

Study	Participants	Ranges of alcohol consumed	Type of alcohol consumed	Results	Conclusions
Marques-Vidal *et al.*, 1995	615 participants from France and Northern Ireland (aged 25-64 years) ECTIM study	0≤15; 15>-≤36; 36>-≤66 and >66 g/day. Non-drinkers comprised the fifth group	Wine, beer, cider and spirits	$PAI\text{-}1_{act}$ (U/ml): non-drinkers: 134 ± 11; 0≤15: 145 ± 9; 15>-≤36:141 ± 11; 36>-≤66:157 ± 10; >66 g/day: 177 ± 11	$PAI\text{-}1_{act}$ levels increased significantly with increased alcohol consumption.
Djousse *et al.*, 2000	1862 participants (men=831, aged 25-91 and women=1031, aged 25-91 years) NHLBI Family Heart Study	Women: never drinkers; ex-drinkers; ≤1.4; 1.5-4.9; 5.0-14.9; ≥15 g/day. Men: never drinkers; ex-drinkers; ≤1.4; 1.5-4.9; 5.0-14.9; 15.0-29.9; ≥30 g/d	Not mentioned	Adjusted means for $PAI\text{-}1_{ag}$ concentrations (ng/mL): women: never drinkers: 10.77; ex-drinkers: 9.41; ≤1.4 g/day: 9.99; 1.5-4.9 g/day: 11.21; 5.0-14.9g/day: 11.28; ≥15 g/d: 16.40. Men: never drinkers: 18.43; ex-drinkers: 15.77; ≤1.4 g/day: 15.19; 1.5-4.9 g/day: 16.20; 5.0-14.9 g/day: 17.27; 15.0-29.9 g/day: 24.02; ≥30 g/d: 29.46	Alcohol consumption up to 14.9 g/d was not associated with increased $PAI\text{-}1_{ag}$ levels among men or women.

Yarnell *et al.*, 2000	2223 male participants of the Caerphilly Study	ml of pure alcohol/week: <11; 11-48; 49-136; 137-229; ≥230	Not mentioned	$PAI\text{-}1_{act}$ %pool: <11: 114.4; 11-48: 118.4; 49-136: 119.0; 137-229: 124.7; ≥230: 137.0	$PAI\text{-}1_{act}$ was positively related to alcohol consumption.
Marques-Vidal *et al.*, 2001	6729 men aged 50-59 years at collaborating centres of the PRIME study (5362 France, 1367 Northern Ireland)	Analysis restricted to drinkers. Total alcohol consumption: France: 318 ± 249 ml ethanol/wk. Ireland: 325 ± 333 ml ethanol/wk. French participants: 0-1890 ml wine/wk; 0-1750 ml beer/wk and 0-1701 ml spirits/wk. Participants from Northern Ireland: 0-661 ml wine/wk; 0-3584 ml beer/wk and 0-2272 ml spirits/wk	Wine, beer and spirits	France: Beer consumption was positively associated with $PAI\text{-}1_{act}$ levels Northern Ireland: Consumption of beer positively associated with $PAI\text{-}1_{act}$. $PAI\text{-}1_{act}$ and consumption of spirits was positively and significantly correlated	Total alcohol consumption in both countries was significantly and positively associated with $PAI\text{-}1_{act}$. Associations differed for different types of alcoholic beverages.
Mukamal *et al.*, 2001	3223 (1456 men and 1767 women) participants from Framingham Offspring study without CVD	Non-drinkers; <3; 3 to <7; 7 to <21 and ≥21 drinks/week.	Beer, wine and liquor	$PAI\text{-}1_{ag}$ (ng/ml) – Women 0 drinks/week: 21.8 ± 16.4; 1-<3:19.8 ± 16.0; 3-<7:18.7 ± 14.3; 7-<21:21.7 ± 17.6. Men (ng/ml) : 0 drinks/week: 23.9 ± 16.0; 1-<3: 24.2 ± 16.6; 3-<7: 22.8 ± 15.6; 7-<21:24.9 ± 18.4; ≥21: 29.0 ± 16.8	$PAI\text{-}1_{ag}$ increased with higher amounts (>7 drinks/wk) of alcohol consumed. Fibrinolytic potential was lower with increasing alcohol consumption. Similar findings for wine, beer and liquor drinkers. Participants consuming wine generally had the lowest $PAI\text{-}1_{ag}$ levels at moderate consumption levels.
Sasaki *et al.*, 2001	203 healthy men aged 26-63 years	Subjects with a weekly alcohol intake of less than 210 g (±16 drinks) were classified as moderate drinkers, and participants with higher intakes as heavy drinkers	Not mentioned	Plasma $PAI\text{-}1_{ag}$ (ng/ml): non-drinkers: 17.3 ± 8.8; moderate drinkers 19.1 ± 10.2; heavy drinkers: 28.3 ± 15.5	Heavy alcohol use increased $PAI\text{-}1_{ag}$ levels in an accumulative fashion. Heavy drinkers had significantly higher $PAI\text{-}1_{ag}$ levels than moderate and non-drinkers. No difference was observed between $PAI\text{-}1_{ag}$ of moderate and non-drinkers.
Volpato *et al.*, 2004	2574 participants from the Health ABC study (men=1256; women=1318) aged 70-79 years	Never; former; occasional (<1 drink/wk); 1-7 drinks/wk; 8-14 drinks/wk and > 14 drinks/wk	Information on specific beverages not collected	Adjusted means for $PAI\text{-}1_{ag}$ (ng/ml): never: 20.5 (1.03); former: 19.7 (1.03); <1: 22.0 (1.03); 1-7: 21.5 (1.03); 8-14: 25.0 (1.07); >14: 26.3 (1.1)	This study did not show any relationship between moderate alcohol consumption and $PAI\text{-}1_{ag}$.

Studies in which PAI-1 and alcohol were not the main focus					
Margaglione *et al.* 1998	1032 Caucasian participants aged 22-66 years (women 586, men 446)	Does not consume alcohol, consumes alcohol,	Not specified	PAI-1_{ag} (ng/ml): Does not consume alcohol: 11.5 (1.89) Consumes alcohol: 13.8 (1.93)	PAI-1_{ag} levels differed significantly between drinking and non-drinking groups with participants consuming alcohol having the higher PAI-1_{ag} value.

CVD: Cardiovascular disease

Moderate levels of alcohol consumption did not seem to affect PAI-1 (Djousse *et al.,* 2000; Mukamal *et al.*, 2001; Sasaki *et al.*, 2001), however, PAI-1 was increased with heavy alcohol consumption (Marques-Vidal *et al.*, 1995; Yarnell *et al.,* 2000; Marques-Vidal *et al.*, 2001; Mukamal *et al.*, 2001 & Sasaki *et al.*, 2001). Djousse *et al.* (2000) found increased PAI-1_{ag} with more than 14.9 g (approximately one drink) alcohol consumed per day, and Mukamal *et al.* (2001) found increased PAI-1_{ag} in participants consuming more than seven drinks per week (one drink per day). Similar results were seen from studies in which PAI-1 was measured as PAI-1_{act} and PAI-1_{ag}.

Plasminogen activator inhibitor-type 1: Intervention (lasting 30 days to twelve weeks)

Study	Participants	Ranges of alcohol consumed	Type of alcohol consumed	Results	Conclusions
Gorinstein *et al.*, 1997	28 males between the ages of 51-73 years. Randomly divided between experimental (n=22) and control group	Experimental group: 330 ml Maccabee beer (contains ± 20 g/alcohol) every day for 30 consecutive days. Control group did not consume any alcohol during this 30-day period	Maccabee beer	Experimental group: baseline: ± 7 ua/ml; 30 days: ± 6.4 ua/ml. Control group: baseline: ± 7.5 ua/ml; 30 days: ± 7.3 ua/ml	PAI-1_{act} remained unchanged and in subjects in whom a reduction in PAI-1 levels was observed the decrease was not significant.
McConnell *et al.*, 1997	19 healthy men and women aged 23-51 years	Participants first abstained from alcohol for two weeks, then followed a six-week intervention during which 13.5 g of ethyl alcohol was consumed daily in the form of Samuel Adams Boston Lager, after which another two weeks of alcohol abstention followed	Beer	PAI-1_{act} (U/ml): during periods of alcohol abstention: 7.1 ± 1.3; Alcohol consumption: 9.3 ± 1.9 PAI-1_{ag} (ng/ml):) during periods of alcohol abstention: 13.3 ± 1.6; Alcohol consumption: 14.9 ± 2.4	No significant changes in PAI-1_{act} or PAI-1_{ag} were observed with alcohol consumption.

Dimmitt *et al.*, 1998	55 male participants aged 20-63 years, overweight (mean BMI= 26.8)	Twelve-week study, with four weeks baseline familiarisation period, followed by four weeks when subjects were randomised either to continue their usual alcohol intake or to reduce their alcohol intake by consuming a low-alcohol beer.	Swan beer (4.9%vol), Swan Special Light (0.9%vol)	$PAI\text{-}1_{ag}$ during low alcohol intake: 23.8 ng/l, usual alcohol intake: 28.7 ng/l	$PAI\text{-}1_{ag}$ was significantly increased with consumption of four standard drinks per day.
Minani *et al.*, 2002	33 Japanese male participants (mean age 36.9 ± 1.1 years)	Subjects habitually consumed alcoholic beverages daily. In this randomised cross-over design 17 participants kept to their usual drinking for a period of three weeks, after which a three-week period followed in which participants reduced their alcohol intake by at least half of habitual amounts. The remaining participants followed the same protocol but in the reverse order	Beer, sake, wine, whisky and other spirits	During usual intake periods plasma $PAI\text{-}1_{ag}$ levels (µg/l): 44.8 ± 3.9; during reduced alcohol periods: 40.8 ± 6.8	Plasma $PAI\text{-}1_{ag}$ did not differ significantly between the habitual and reduced alcohol intake periods.
Mezzano, 2004	Two groups of healthy males (aged 22 ± 3.4 years)	Intervention consisted of 90 days during which one group followed the Mediterranean diet (MD) for the first 30 days, the MD supplemented with 240 ml/day of red wine for the next 30 days, and in the last 30 days followed only the MD. The second group followed the same protocol, but consuming high fat diets (HFD)	240 ml red wine every day for 30 days	The consumption of red wine led to significant increases in $PAI\text{-}1_{ag}$. For HFD participants, PAI-1 levels increased from ± 28 ng/dL to ± 36 ng/dL. In the MD group it increased from ± 22.5 ng/dL to ± 29 ng/dL	Moderate consumption of red wine with a HFD and the MD increased plasma $PAI\text{-}1_{ag}$ levels significantly.
Avalone *et al.*, 2006	48 apparently healthy participants aged 35-65 years (men=28, women=20)	Participants were assigned to two groups. Group A (n=24) received 250 ml red wine with meals for four weeks, after which they returned to their usual wine intake. Group B followed the same protocol but in the reverse order	12 participants of each group received Nero d'Avola and 12 Etna Torrepalino	Group A $PAI\text{-}1_{ag}$ (ng/ml) Baseline: 51.0 ± 17.7; +4 weeks: 78.9 ± 18.1; +8 weeks: 61.4 ± 20.3. Group B $PAI\text{-}1_{ag}$ (ng/ml). Baseline: 50.0 ± 13.7; +4 weeks: 52.9 ± 14.1; +8 weeks: 81.9 ± 17.3	In this study the supplementation of the diet with red wine induced in group A at four weeks, and in group B at eight weeks (after four weeks of alcohol consumption) significantly increased $PAI\text{-}1_{ag}$ levels.

BMI: Body mass index

Most intervention studies investigating the effect of alcohol consumption on PAI-1 antigen over 30 days to twelve weeks show increased PAI-1 antigen with moderate (women 1-2, men 2-3 drinks per day) alcohol consumption (Dimmit *et al.,* 1998; Mezzao *et al.,* 2004 & Avelone *et al.,* 2006). Studies on PAI-1 antigen in which small amounts of alcohol (13.5 g/day) were consumed and usual alcohol intake was halved, however, did not show significant changes in PAI-1 antigen (McConnell *et al.,* 1997; Minani *et al.,* 2002). Gorinstein *et al.* (1997) and McConnell *et al.* (1997) measured PAI-1_{act} and did not find significant changes in PAI-1_{act} with alcohol consumption.

Plasminogen activator inhibitor-type 1: Acute intervention studies (post-prandial type)

Study	Participants	Ranges of alcohol consumed	Type of alcohol consumed	Results	Conclusions
Veenstra *et al.,* 1990	Two groups of healthy men, one group n=8 (aged 20-30 years), other group n=8 (aged 45-55 years) Average habitual alcohol intake was between 10-40 g/day	Experimental period consisted of eight weeks during which each participant had one experimental day every two weeks. On these experimental days participants would ingest either a rich protein dinner, with or without alcohol, or a high fat dinner with or without alcohol. During the experimental period participants continued their habitual food and alcohol consumption, except for experimental days, when drinks and meals were supplied.	Red port and two glasses of red wine (total alcohol=30 g)	Blood for PAI-1_{act} analyses was collected one hour after dinner and 15 hours after alcohol consumption. PAI-1 levels (%). Younger group: Without alcohol: ± 67, with alcohol: ± 90. Older group: Without alcohol: ± 74, with alcohol: ± 115	In the older group PAI-1_{act} was increased significantly with alcohol consumption, in the younger group, however, this alcohol-induced rise in PAI-1_{act} levels was not significant.
Hendriks *et al.,* 1994	Eight healthy men aged 45-55 years, with normal BMI Habitual alcohol consumption was moderate (1-4 glasses/day)	The study period consisted of 11 days during which there were four experimental days. On experimental days participants stayed overnight at a metabolic ward. Participants received a standard diet during these 11 days; during the experimental days these meals were supplemented with either 400 ml carbonated mineral water, 40 g alcohol in the form of 1000 ml beer, 400 ml red wine, or 144 ml spirits.	Red wine, beer and spirits	Consumption of either beer, wine or spirits resulted in PAI-1_{act} increasing from 53 ± 19% before dinner to 667 ± 283% five hours after dinner	Regardless of the type of beverage consumed, PAI-1_{act} continued to increase up to five hours after moderate alcohol consumption with dinner, but returned to normal by the following day.

El-Sayed *et al.*, 1999	11 healthy, moderately trained participants (mean age 22.8 ± 1.4 years)	Participants each performed two exercise trials which were separated by seven days. 45 min before the first trial participants consumed 0.5 g/kg vodka diluted 1 in 4 with a glucose-free orange flavoured solution. On the second trial an equal volume of non-alcohol glucose-free orange-flavoured solution was ingested. Participants then cycled continuously at 65% VO2max for 30 min, followed by an all-out self-paced performance of 5 min. Blood samples were collected 45 min after ingestion of beverage and immediately after exercise.	Vodka	PAI-1_{act} (AU/ml): Control trial: Rest: 15.7 ± 1.2; 45 min after ingestion: 14.2 ± 1.1; After exercise: 6.2 ± 1.3. Alcohol trial: Rest: 15.8 ± 1.3; 45 min after ingestion: 14.4 ± 1.4; After exercise: 5.3 ± 1.3	PAI-1_{act} decreased significantly from before exercise and 45 minutes after ingestion of beverage in both the control and experimental groups, thus the decrease on PAI-1_{act} may be due to the exercise and not the ingestion of alcohol.
Numminen *et al.*, 2000	20 healthy men, participants aged 20-39 years	Three sessions, of which two were ethanol and one a control session, were participated in. Participants spent experimental days in a controlled environment. Half-hourly drinks containing either fruit juice, or an equal volume of the fruit juice mixed with ethanol (2 mol/L solution) were consumed over a time span of 2½ hours.	Ethanol mixed with fruit juice (not including grape juice)	Results from experimental trials. PAI-1_{act} in (U/ml) 7pm: 6; 10pm: 19; 01am: 34; 04am: 32; 07am: 14. 08am: 4; 11am: 24; 02pm: 37; 05pm: 23; 08pm: 10 Baseline, PAI-1_{act} in (U/ml) 7pm: 3; 10pm: 3; 01am: 8; 04am: 7; 07am: 8. 08am: 8; 11am: 8; 02pm: 10; 05pm: 8; 08pm: 4	A 2 mol/L solution ethanol and fruit juice drunk half-hourly for 2½ hours lead to a significant increase in PAI-1_{act}. This increase is not dependent on circadian patterns. Alcohol induced increase peaked approximately three hours after discontinuation of drinking, after which PAI-1_{act} started to decrease and continued to decrease rapidly until the end of the session.
Delahousse *et al.*, 2001	Ten male alcoholic participants were admitted to a drying-out centre (27-55 years) Consuming 100-548 g ethanol/ day on admission to the centre	Participants started with withdrawal therapy the day after admission (D1), and no alcohol was allowed for the remainder of the therapy period. Blood samples were collected for D1 and D22.	Usual alcohol consumption of participants included wine, beer and spirits	PAI-1_{act} for D1: 25 ± 10AU/ml; for D22: 17 ± 9AU/ml	PAI-1_{act} and functional levels decreased significantly over the course of 22 days in most participants with abstention.

Sierksma *et al.*, 2001	Six pre-menopausal women using oral contraceptives, six post-menopausal women. Habitual daily alcohol consumption range between one and two drinks	One-week run-in-trial during which participants consumed three glasses (120 ml/glass) of grape juice before and during dinner. Thereafter half of the group entered a period of three weeks during which three glasses (30 g alcohol/d) of red wine were ingested before and during dinner; the other half continued drinking red grape juice. After three weeks the participants followed the same protocol, only *vice versa.* After each three-week period participants spent the night in a controlled environment where blood samples were collected.	Red wine ("Mon detour", Châeau sablay 1993, 11.5% alcohol). Red grape juice ("Fruitdruifje", Riedel Drankenindustrie , Ede, The Netherlands)	A sharp increase of PAI-1_{act} was seen in post-menopausal women up to 5h after dinner. In this group PAI-1_{act} was significantly higher after consumption of wine, compared to grape juice. After 13h, PAI-1_{act} returned to basal levels. There were no differences between PAI-1 values obtained for wine and grape juice in the pre-menopausal group	Moderate alcohol consumption with dinner increased PAI-1_{act} levels of post-menopausal women significantly five hours after consumption, but it returned to normal the following day.
Van de Wiel *et al.*, 2001	50 healthy male volunteers aged 26 ± 4.6 years participated in this study. Habitual alcohol intake was two units of an alcoholic beverage per day.	Participants abstained from alcohol for one week before the experiment. Blood samples were collected before and during the experiment. 1st Experiment: 12 participants consumed two glasses (250 ml) red wine; 18 consumed six glasses (750 ml) red wine; controls ingested mineral water with their meal. 2nd Experiment: six participants consumed four glasses (500 ml); six consumed eight glasses (1000 ml) of red wine and four participants consumed mineral water.	Spanish Valdepenas red wine (Diego de Almagro 1994, 12.5 vol% alc)	1st Experiment (mean PAI-1_{act} values in U/mL) 250ml wine: 1500h: 4 ± 1; 2300h: 7 ± 4; 0900h: 7 ± 7. 500ml wine: 1500h: 3 ± 4; 2300h: 28 ± 8; 0900h: 12 ± 9. Mineral water (both experiments): 1500h: 9 ± 3; 2300h: 7 ± 4; 0900h: 11 ± 6. 2nd Experiment (mean values in U/ml) 750 mL wine: 1500h: 8 ± 3; 2300h: 34 ± 16; 0900h: 14 ± 9; 1000 mL: 1500h: 2 ± 1; 2300h: 27 ± 16; 0900h: 23 ± 27	PAI-1_{act} increased maximally five hours after ingestion of 250, 500, 750 and1000 ml red wine. Parameters were again normal the next morning, except for participants who consumed 1000 ml wine, for whom the PAI-1_{act} was still elevated.
Tousoulis *et al.*, 2008	83 healthy young participants assigned to five groups in which equal amounts of alcohol were consumed	Each beverage contained 30 g of alcohol. 264 ml red wine, 264 ml white wine, 633 ml beer or 79 ml whisky; the last group ingested 250 ml water. Blood samples were collected at baseline and 4h after consumption.	Red and white wine, beer and whisky	PAI-1_{ag} concentrations (ng/ml): Red wine - baseline: 17.8 ± 3.6, 4h: 23.9 ± 3.9; white wine - baseline: 15.2 ± 2.99, 4h: 25.1 ± 4.8; beer – baseline: 15.8 ± 3.11, 4h: 24.5 ± 5.93; whisky – baseline: 11.1 ± 1.3, 4h: 22.6 ± 4.9; water – baseline: 17.9 ± 3.09, 4h: 27.9 ± 4.7	PAI-1_{ag} levels were significantly increased 4h after ingestion of various individual beverages.

Kiviniemi *et al.*, 2009	22 healthy Finnish men aged 23 ± 1.8 years, subjects were non-smokers, normolipidemic and non-diabetic	1.0 g/kg ethanol from either red wine or cognac or an equal volume of dealcoholised red wine in a randomised cross-over study with a washout period. PAI-1measured before and 1-2 h after ingestion	Parlage Cabernet del Veneto 2003, Ebony Vale Cabernet Sauvignon 2003 (dealcoholised), Remy Martin VSOP	PAI-1 (µg/l): Red wine before: 9.06 ± 4.99, after: 18.7 ± 11.47; dealcoholised red wine before: 7.35 ± 3.51, after: 6.13 ± 2.21; Cognac before: 8.32 ± 4.14, after: 9.82 ± 7.80. Significant differences in PAI-1 were found between red wine and dealcoholised red wine; between red wine and cognac; but not between dealcoholised wine and cognac	1-2 h after beverage consumption PAI-1 was significantly increased when red wine was drunk. Dealcoholised red wine and cognac did not change PAI-1 significantly.

BMI: Body mass index

Studies in which PAI-1_{act} was measured after moderate alcohol consumption showed an increase in PAI-1_{act} lasting approximately three to five hours, after which PAI-1_{act} started to decrease and return to normal by the following day in studies which continued measurements (Veenstra *et al.*, 1990; Hendriks *et al.*, 1994; Numminen *et al.*, 2000; Sierksma *et al.*, 2001; Van de Wiel *et al.*, 2001). In the study of Van de Wiel *et al.* (2001), the PAI-1_{act} levels of participants who consumed 1000 ml wine were still elevated the following morning. Veenstra *et al.* (1990) and Sierksma *et al.* (2001) compared groups of different ages. Veenstra *et al.* (1990) found PAI-1_{act} to increase significantly in a group of men aged 45-55 years, but no significant increase was seen in the group aged 20-30 years. Sierksma *et al.* (2001) found that PAI-1_{act} increased significantly for five hours after consumption of alcohol with dinner in a group of post-menopausal women, but no significant changes were observed in pre-menopausal women. These results indicate that differences in participant selection may influence the effect alcohol has on PAI-1. Only one study measured PAI-1_{ag} (Tousoulis *et al.*, 2008) and the results also show increased PAI-1_{ag} levels four hours after alcohol consumption.

2.6. ALCOHOL, PAI-1 AND FIBRINOGEN IN BLACK AFRICANS

2.6.1. Transition (drinking patterns in the black South African population)

The black population in South Africa is undergoing rapid transition with evidence of increasing alcohol consumption and therefore they appear to be at particular risk for alcohol-related problems (Strachan, 1999; Vorster, 2002). Informal and unregulated alcohol brewing and sales as well as poverty contribute to the problem (Strachan, 1999). In the South African demographic health survey of 2003 a higher percentage of African urban men and women reported having ever used alcohol (men 48%; women 20.3%) as well as having used alcohol within the preceding seven days (men 31.2%; women 8.5%) in comparison with the survey conducted in 1998. Among black African men, 38.7% of urban men as opposed to 29.5% rural men reported drinking alcohol in the previous twelve months (DOH, 2003). Among black African women, 14.2% of urban and 7.5% of rural women had consumed alcohol in the previous twelve months (DOH, 2003). Thus, it seems that higher proportions of urban Africans than their rural counterparts consume alcohol. Of particular concern is the pattern of binge drinking (defined as drinking five or more and three or more standard drinks per day for men and women respectively as used in the SADHS, 1998) observed in the African population mostly over weekends (Parry *et al.,* 2005).

2.6.2. Differences in PAI-1_{act} and fibrinogen concentration between black Africans and Caucasians

PAI-1 has been shown to be significantly lower, but still within reference range, in black Africans when compared with Caucasians (Jerling *et al.,* 1994; Festa *et al.*, 2003; Pieters *et al.,* 2006; Greyling *et al.*, 2007; Nienaber *et al.*, 2008). Greyling *et al.* (2007) found plasma PAI-1_{act} levels in Caucasian participants to be twice as high as the PAI-1_{act} in the African participants.

Evidence is emerging that suggests that the low levels of PAI-1 observed in black Africans may be partly due to the 4G/5G genetic polymorphism discussed in section 4.4. Data from the United States indicate that among blacks the 4G-allele is less common than in Caucasians (Crainich *et al.*, 2003; Festa *et al.*, 2003). The only data

available regarding Africans come from a small case-control study on pre-eclampsia conducted among South African Zulu women, in which the prevalence of the 5G-allele in the controls was even higher (0.88) than generally observed in African Americans (about 74%) (Pregoraro *et al.*, 2003). These genotype-related differences in PAI-1 concentration were, however, not present in all studies (Henry *et al.*, 1997; Cesari *et al.*, 1999). More recently, evidence is accumulating that while the effect of this polymorphism on basal PAI-1 levels may still be somewhat unclear, it may affect PAI-1 responses (Dawson *et al.*, 1993; Eriksson *et al.,* 1998; McCormack *et al.*, 1998; Menges *et al.*, 2001). This implies that the difference in PAI-1 levels between 4G and 5G becomes more obvious in the presence of environmental (such as alcohol) and/or disease factors which stimulate PAI-1 expression.

In general, higher levels of fibrinogen have been observed in black Africans compared with levels typically observed in healthy Caucasians, but the reason for this is unknown (Vorster *et al.*, 1998; Vorster, 2002). The question that now arises is how alcohol consumption influences fibrinogen levels in a population such as black South Africans, who already present with high fibrinogen levels in the healthy.

2.7. CONCLUSION

Epidemiological studies in which the populations observed were mostly Caucasian generally show a negative correlation for fibrinogen concentration with moderate alcohol consumption, as well as increased PAI-1_{act} and antigen levels with heavy alcohol intake. Very little information regarding PAI-1_{act}, fibrinogen and their association with alcohol consumption in black South Africans is available. Furthermore, PAI-1_{act} levels have been shown to be lower and fibrinogen concentrations to be higher in black African populations when compared with Caucasians. Additionally, in South Africa the black population is undergoing a process of urbanisation, which is associated with changes in diet and physical activity that seem to have a negative influence on body composition and lipid levels. The remainder of this dissertation will focus on the association between

alcohol, PAI-1_{act} and fibrinogen, and how factors such as gender, urbanisation, waist circumference, BMI, as well as triglycerides and the 4G/5G polymorphism in the case of PAI-1_{act}, may influence the association between alcohol consumption and PAI-1_{act} and fibrinogen levels in black South Africans.

CHAPTER 3: METHODS

3.1. INTRODUCTION

While the association between alcohol consumption, PAI-1 and fibrinogen concentration in Caucasians has been studied extensively, much less is known about this association in black Africans. The aim of this study was to determine the association between the habitual alcohol consumption of participants of the PURE study and the PAI-1_{act} and fibrinogen concentrations of these participants, as well as to determine whether factors known to influence PAI-1 and fibrinogen, may modify this relationship. Specific objectives were:

- To investigate the possibility of GGT and %CDT use as proxy markers in the association between alcohol, fibrinogen and PAI-1_{act}
- To investigate the effect of urbanisation on the association between alcohol consumption, PAI-1_{act} and fibrinogen
- To investigate the association between habitual alcohol consumption and PAI-1_{act} in relation to the 4G/5G polymorphism
- To investigate the association between habitual alcohol consumption and PAI-1_{act} and fibrinogen concentration in relation to body composition (BMI and waist circumference)
- To investigate whether the association between habitual alcohol consumption and PAI-1_{act} is modulated by triglyceride concentration.

In this chapter the recruitment and characteristics of participants as well as the experimental methods used are described. Although the data collection and analysis of blood samples were finalised before the beginning of the present study, all the statistical analysis and writing up of data were performed by the student of the present study. Blood samples were analysed by qualified technicians in an accredited research laboratory at the Vaal University of Technology, except for PAI-1_{act} and the 4G/5G

polymorphism analyses which were performed at the Nutrition laboratory of the School of Physiology, Nutrition and Consumer Science of the North-West University, Potchefstroom Campus. DNA sequencing was done at the Central Analytical Facility of the University of Stellenbosch. Results will be reported in Chapter 4, which will then be discussed in Chapter 5.

3.2. ETHICAL CONSIDERATIONS

Ethical approval was obtained from the Ethics Committee of the North-West University (Ethics number 04M10, date of ethical approval 14 June 2005). In rural areas, permission to recruit participants was obtained from community leaders and tribal chiefs. The study and all study procedures were explained to participants in their home language before informed consent was obtained. Participants had the option of withdrawing at any stage during the study.

3.3. STUDY POPULATION

3.3.1. Recruitment

A census of 6000 African households was undertaken, starting from a randomly selected address in each of four (two rural and two urban) communities in the North West province, South Africa. The populations were considered to be stable and, in the case of rural populations, not susceptible to migration of large groups to urban areas. Fieldworkers visited 1500 households in each community. During this visit the head of each household willing to participate in the South African arm of the Prospective Urban and Rural Epidemiological (PURE) study completed a demographic questionnaire after providing informed consent. After the census, a total of 3750 questionnaires had been completed. From these completed questionnaires the 2010 apparently healthy volunteers who did not report the use of medication and with no reported chronic disease or known HIV infection were included in the study.

Approximately 1000 subjects were recruited from the rural (urban-rural: Ganyesa, and very-rural: Tklagameng) and 1000 from the urban (established urban: Ikageng, and squatter camps: Zonderwater extensions 7 and 11) areas. Clinic staff, local ward communities, churches, chiefs and local municipalities were facilitators in the organisation process of this study in the various communities.

3.3.2. Inclusion criteria

Volunteers between the ages of 35 and 60 years were eligible to participate in the PURE study. Household members (usual and migrant residents) of rural and urban households were included in this study. Household members were defined as usual residents: a person who eats and sleeps in a household on most days of the week and most weeks of the year, and who considers the household his/her primary place of habitation over the long term. 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/sleep elsewhere owing to the need to work, study or train in a different physical location. 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.

3.4. STUDY DESIGN

The PURE study is a large-scale cohort study that aims to track changing lifestyles, risk factors and chronic disease, using periodic standardised data collection in urban and rural areas of 17 low-, middle- and high-income countries from around the world for at least ten years. Data are collected on national, community, household and individual level (Teo *et al.*, 2009).

During the baseline measurements, each participant completed the standardised interviewer-based adult questionnaire, the dietary frequency questionnaire (discussed in section 3.5) and a physical activity questionnaire (data not used in the present study); as previously mentioned (section 3.2.3), non-responder questionnaires were completed by fieldworkers when the head of a household was not available or did not want to participate in the study. Blood samples were collected (section 3.7) and anthropometric measurements (section 3.5) of each participant were also taken. The present study investigated the habitual alcohol consumption within the South African PURE cohort and its association with PAI-1_{act} levels and fibrinogen concentration in this population, using the baseline data of the 2010 subjects, collected over a period of twelve weeks in 2005.

3.5. ANTHROPOMETRICAL ASSESSMENT

All anthropometrical assessments were done using the guidelines adopted at the NIH-sponsored Arlie Conference (Lohman *et al.*, 1988: 3, 8, 45, 46, 49, 52, 66 & 67). Weight, height, hip circumference, waist circumference, mid-upper arm circumference, triceps skin fold, right calf circumference and skin fold were measured in the study population. Subjects were examined wearing minimal clothing. Weight in kilograms was measured with participants not wearing shoes, using a portable electronic scale (Precision Health Scale, A&D Company, Tokyo, Japan). A stadiometer (IP 1465, Invicta, London, UK) was used to measure height (in metres) with the subjects standing upright, without shoes and their heads in the Frankfort plane. BMI was calculated by dividing weight by height squared. John Bull callipers (British Indicators, London, UK) were used to measure skin folds, using the appropriate landmarks. Circumferences were measured using the appropriate landmarks to the nearest 0.1 cm, with a non-stretchable standard tape measure (Lufkin, Cooper Tools, Apex, NC, USA).

Blood pressure was measured by a trained fieldworker with the subject sitting upright and relaxed with his/her right arm supported at heart level. Before this measurement was taken, subjects had to have been resting and calm for more than five minutes, and should not have climbed stairs, exercised or smoked within 30 minutes before the taking of the measurement.

3.6. DIETARY INTAKE ANALYSIS

Interviewer-based quantitative food frequency questionnaires (QFFQ) were completed to determine the dietary intakes as well as the habitual alcohol consumption of participants. Participants indicated the volume of beverage consumed each time alcohol was consumed as well as the type of beverage consumed. Total daily alcohol intakes of alcoholic beverages were calculated by dividing by 30 the total amount of alcohol reported to be consumed on a QFFQ, (participants reported alcohol consumption for the previous month). Alcohol consumption is also expressed as gram ethanol per day by taking the alcohol content of the beverages into consideration. Standard alcoholic beverages such as 340 ml malt beer, 340 ml cider, 25 ml brandy, whiskey, gin or vodka, and a 120 ml glass of wine each contain approximately 12 g ethanol, which was the amount used to determine daily ethanol intake (Wolmarans *et al.*, 1992). The *Foodfinder3*® program (Medical Research Council, Tygerberg, 2007) was used to computerise the dietary data, which were then sent to the Medical Research Council of South Africa for nutrient analyses.

3.7. BLOOD SAMPLING AND ANALYSES

A 90 ml fasting blood sample was collected by a qualified nursing sister from the antecubital vein in the right arm of each subject with the use of a disposable needle. Each collection tube was filled to capacity to ensure optimal blood anticoagulant ratios

and gently inverted five times to ensure thorough mixing of contents. The tube was then placed in an ice box.

For the determination of fibrinogen concentrations and PAI-1_{act}, blood was collected in 3.8% citrate tubes. The collection tube was then put in an ice box until processing. Samples were centrifuged at 2000 x *g* for 15 minutes at 10 °C within two hours of collection and stored in bio-freezers at -82 °C until further analysis. For the measurement of GGT, %CDT, high sensitivity C-reactive protein (CRP) and triglycerides, blood was allowed to clot at room temperature for 30 minutes and centrifuged at 2000 x *g* for 15 minutes at 10 °C, and serum samples were stored at -82 °C until analysis. For the determination of 4G/5G genotype, citrated whole blood was centrifuged at 10 °C for 15 minutes at 2000 x *g* and the buffy coat transferred to storage tubes which were stored at -82 °C until deoxyribonucleic acid (DNA) isolation and further analysis.

3.7.1. Determination of fibrinogen concentration

Plasma fibrinogen concentrations were measured using a modified Clauss method with the Multifibren U test kit on the Dade Behring BCS coagulation analyser. The principle of the method used is the coagulation of citrated plasma by a large excess of thrombin. The amount of fibrinogen in the specimen determines the coagulation time. A fibrinogen calibrator kit is used to calculate the reference curve, and the fibrinogen concentration is given in g/l (Dade Behring, Deerfield, USA).

3.7.2. Determination of PAI-1_{act}

A chromogenic assay kit was used for the determination of PAI-1. This is done by adding a fixed amount of t-PA to a plasma sample, which is then allowed to react with PAI-1 present in the sample. The sample is then acidified to destroy potential plasmin inhibitors, and subsequently diluted. A mixture of Glu-plasminogen, poly-D-lysine and chromogenic substrate is added. The quantity of active t-PA remaining in the sample will catalyse the conversion of plasminogen to plasmin, which will then hydrolyse the chromogenic substrate. The amount of colour which develops is proportional to the

amount of active t-PA in the sample. The PAI-1_{act} content in the sample is the difference between the amount of t-PA added to the sample and the amount of t-PA recovered (Spectrolyse pL PAI-1, Biopool, Trinity Biotech, Ireland).

3.7.3. Determination of gamma glutamyl transferase (GGT)

GGT were analysed with the Sequential multiple analyser computer (SMAC) using the Konelab™ auto analyser, which is a clinical chemistry analyser used for colorimetric, immunoturbidimetric and ion-selective electrode measurements. The Konelab™ kit used works on the principle that GGT catalyses the transfer of glutamic acid to acceptors like glycylglycine. During this process 5-amino-2-nitrobenzoate is released, which absorbs light at a wavelength of 405 nm, the increase in absorbance being directly related to GGT activity (Thermo Electron Corporation, Vantaa, Finland).

3.7.4. Determination of %CDT

An *in vitro* heterogeneous immunoassay with column separation followed by turbidimetric measurement was used to measure %CDT in this population. Serum transferrin in a sample is saturated with Fe^{3+}, after which the mixture is applied to an ion-exchange column. Transferrin isoforms carry different charges and are separated in the column. The CDT isoforms are eluted and the content of the collected elute is determined by turbidimetric measurement. Immune complexes are formed by the eluted CDT isoforms and anti-transferrin antibodies. These anti-transferrin antibodies are used to measure the total transferrin content of the sample. Measurements are evaluated using a calibration curve, and the %CDT value is calculated (Axis-Shield %CDT kit, Oslo, Norway).

3.7.5. Determination of triglyceride concentration

Triglycerides were also measured with a SMAC, using the Konelab™ auto analyser. The principle on which this procedure works is that lipase hydrolyses triglycerides to glycerol and fatty acids. Glycerol is phosphorilated to glycerol-3-phosphate, which is oxidised to dihydroxyacetone phosphate and hydrogen peroxide. A quinoneimine dye is formed when the hydrogen peroxide reacts with 4-aminoantipyrine and 4-chlorophenol.

The absorbance is measured at 510 nm. The triglyceride concentration is subsequently calculated by using a calibration curve (Thermo Electron Corporation, Vantaa, Finland).

3.7.6. Determination of high sensitivity C-reactive protein

High sensitivity CRP was measured with a SMAC, again using the Konelab™ auto analyser. Micro-particles coated with anti-human CRP are added to buffered samples. When the reaction between these CRP-coated micro-particles and the samples has reached its end-point, the immunoprecipitation is measured at 540 nm. The change in absorbance is proportional to the amount of CRP in the solution (Thermo Electron Corporation, Vantaa, Finland).

3.7.7. DNA isolation and genotyping

DNA isolation

The QIAGEN Flexigene DNA Extraction kit was used for DNA isolation. In this procedure, lysis buffer was added to 400 µl buffy coat from each participant. After protein digestion with protease, DNA was precipitated by adding isopropanol, recovered by centrifugation, washed with 70% ethanol and dried. Thereafter, DNA was re-suspended by the addition of a hydration buffer (Qiagen, Duesseldorf, Germany).

4G/5G genotyping

For the 4G/5G genotyping, a representative sequence (NT-007933) on the National Centre for Biotechnology Information (NCBI) website was used for the PAI gene. From this sequence a primer pair was designed to amplify nearly the entire PAI promoter region of the PAI gene. A polymerase chain reaction (PCR), in which the designed primer pairs were used, was used to amplify the DNA of 30 randomly chosen PURE participants. Post-PCR cleanup and the sequence reactions were performed in part by the North-West University Nutrigenetics Laboratory personnel and the Central Analytical Facility of the University of Stellenbosch. The sequencing electrophoresis (sequencing was done in both directions) was done at the Central Analytical Facility (Stellenbosch, South Africa) on an automated DNA sequencer using cyclic sequencing. Sequences were then entered into the BioEdit sequence alignment editor (version 7.0.9.0),

manually checked and aligned with the reference sequence. The computer-generated alignments were refined manually. From these sequences it was determined which of the 30 participants presented with 4G or 5G homozygotic alleles or the 4G/5G heterozygotic allele. These participants were used as controls in further analyses.

Primers were designed to flank the 4G/5G polymorphism (See Table 3.1). This sequence was designed to be smaller than 200 base pairs. The primers were synthesised commercially (IDT, Coralville, USA). These primers were tested with conventional PCR, and the PCR products were run on a 2% (w/v) agarose gel to verify whether the correct fragment size was obtained. PCR amplification of a PAI gene promoter was performed in a 25 µl (total volume) reaction mixture containing 50 ng DNA, 1 x Taq DNA Polymerase (Promega, Madison, USA), each deoxynucleotide triphosphate at a concentration of 0.2 mM, 0.5 µM Primer Reverse, 0.5 µM Primer Forward and 1.5 mM MgCl2. Temperature gradients were used to determine the optimal annealing temperature. The optimal annealing temperature for the PCR (where the fragments were most visible on the agarose gel) was found to be 63 °C. Subsequently, probes were commercially synthesised (see Table 3.1).

Two probes (Tjarlünd *et al.*, 2003) specific for the 4G and the 5G single nucleotide polymorphism (SNP) were synthesised commercially (IDT, Coralville, USA). Optimisation was done with both probes and primers in the PCR reaction mixture until the reaction was specific enough for the Bio-Rad software to distinguish between the two alleles. The optimal concentrations of the primers and probes that were used were: 600 mM forward primer, 600 mM reverse primer and 200 mM probe.

Real-time PCR amplification was performed in a 15µl (total volume) reaction mixture containing 1 x Sensimix (Qiagen, Duesseldorf, Germany), 600 mM Primer, 3 mM MgCl2, 600 mM Primer 4G/5G – F, 600 mM Primer 4G/5G – R, 200 mM Probe 4G, and 200 mM Probe 5G.

Table 3.1. Primers and probes used to determine the 4G/5G genotype

	Name	Sequence	Melting temperature (Tm)	GC content
Primers that were used to amplify entire PAI gene	PAI_1_F	5' – TTC CAC CCA CTG AAA CTT CC – 3'	55.0 °C	50.0%
	PAI_1_R	5' – GAT GGG AGA CCG TGA CAG AT – 3'	56.3 °C	55.0%
	PAI – 2F	5' – GGT TGC AAG CTC CCT ATG AG – 3'	55.7 °C	55.0%
	PAI – 2R	5' – CAG CCA CGT GAT TGT CTA GG – 3'	55.5 °C	55.0%
	PAI – 3F	5' – GGG AGT CAG CCG TGT ATC AT – 3'	56.6 °C	55.0%
	PAI -3R	5' – AGT TCT CAG AGG TGC CTT GC – 3'	57.3 °C	55.0%
Primers	Forward: 4G/5G2 – F	5' – TCT TTC CCT CAT CCC TGC C – 3'	56.8 °C	57.8%
	Reverse: 4G/5G2 – R	5' – CCA ACC TCA GCC AGA CAA GG – 3'	58.2 °C	60%
Probes	4c probe: PAI_4C	5' - /5HEX/ACA CGG CTG ACT CCC CAC GT/ 3BHQ_1/ - 3'	63.7 °C	65%
	5C probe	5' - /56 – FAM/ACG GCT GAC TCC CCC ACG T/ 3BHQ_1/ -3'	64.1 °C	68.4%

Amplification was carried out with the Bio-Rad IQ5 PCR thermal cycler on 96 well plates, each plate containing a control for each allele as well as three no-template (blank) controls distributed throughout the plate. The initial denaturation step (10 minutes at 95 °C) was followed by five cycles of PCR, with each cycle consisting of a denature step of 30 seconds at 95 °C, an annealing step of 30 seconds at 63 °C and an extension step of 30 seconds at 72 °C, followed by a final elongation step of 70 minutes at 72 °C. Synthesis of the products was monitored after each extension step at 72 °C by measuring the fluorescence of each dye (FAM and HEX), using the IQ5 iCycler (Bio-Rad, Hercules, USA).

Using the Bio-Rad IQ5 optical system software, allelic discrimination was used to distinguish between homozygote 4G/4G, heterozygote 4G/5G and homozygote 5G/5G. In cases where discrimination was not clear, amplification and analysis were repeated.

3.7.8. HIV testing

For the voluntary HIV test, participants received pre-test counselling. During this counselling session participants were given information on what the human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) are, how the disease is transmitted, test procedures, how confidentiality will be ensured, and the use of anti-retroviral therapy. On-site testing was done according to the South African Department of Health protocol and the UNAIDS/WHO Policy Statement on HIV-testing (UNAIDS/WHO: 2004). The Rapid HIV test was used and, if positive, a Pareeshak test was performed to confirm the results. For participants who chose to know the results of their test, post-test counselling was done in privacy. Counsellors were available to participants if necessary, and follow-up plans were made according to the test results.

3.8. STATISTICAL ANALYSIS

Data were analysed with the computer software package Statistica® (Statsoft Inc., Tulsa, Oklahoma, USA). A *P*-value of less than or equal to 0.05 was regarded as statistically significant. Data were tested for normality. Parametric data were expressed as the mean (95% confidence intervals (CI)). Non-parametric data were log-transformed to improve normality and are reported as geometric mean (95% confidence intervals). Owing to the large number of participants who had PAI-1_{act} of 0 U/ml (calculated from the standard curve of the assay), a value of 1 was added to all PAI-1_{act} values before log transformation. This was subtracted again when reporting the geometric mean and CI. Spearman correlations were used to determine the association of alcohol intake with PAI-1_{act}, and fibrinogen concentration. T-tests and analysis of variance (ANOVA) were used to compare PAI-1_{act} and fibrinogen concentration in

different sub divisions of the populations. Analysis of co-variance (ANCOVA) was used to take possible confounders into consideration. Adjusting for confounders did not significantly change the means and only the ANCOVA p-values are reported. In order to determine which confounders to adjust for, correlations were used. Variables that significantly correlated with PAI-1_{act} (waist circumference and triglycerides) or fibrinogen (CRP) were treated as confounders, and were adjusted for. No adjustment was made for age as there was no correlation between age and PAI-1_{act} and fibrinogen. There was also no difference in fibrinogen concentration between current smokers, non-smokers and previous smokers and therefore no adjustment or stratification was done for smoking. Participants were divided into three drinking categories: non-drinkers, moderate drinkers (men who consume less than 30 g alcohol per day, women who consume less than 15 g alcohol per day), and heavy drinkers (men who consume 30 g and more alcohol per day, women who consume 15 g and more alcohol per day). The population was sub-divided according to: urbanisation, BMI, waist circumference, 4G/5G polymorphism and triglyceride concentration (for PAI-1) and gender. In order to determine the influence of these sub-divisions on the association between alcohol consumption and PAI-1_{act} and fibrinogen concentration, two-way ANCOVA was used and the interaction p-values reported in Table 4.3 and 4.4 in the results chapter. Multiple regression (forward stepwise) was used to determine the predictive value of alcohol and population sub-divisions to PAI-1_{act} and fibrinogen levels compared with other variables.

CHAPTER 4: RESULTS

4.1. INTRODUCTION

This chapter presents the baseline characteristics of the PURE study population, population characteristics of different drinking categories, the association between alcohol, PAI-1_{act} and fibrinogen, as well as the influences of various population characteristics on the association of alcohol consumption and PAI-1_{act} and fibrinogen in the South African PURE population.

4.2. DETERMINATION OF CONFOUNDERS INFLUENCING THE ASSOCIATION BETWEEN ALCOHOL CONSUMPTION AND PAI-1_{ACT} AND FIBRINOGEN

Before investigating the association between alcohol consumption and PAI-1_{act} and fibrinogen, it is necessary to determine whether there are possible confounders that may influence this association and which would have to be adjusted for.

The literature shows age to be a confounder for fibrinogen, and smoking to be a confounder for both PAI-1 and fibrinogen. However, age did not correlate strongly with fibrinogen in this population ($r = 0.16$). PAI-1_{act} and fibrinogen also did not differ significantly ($p = 0.99$ and $p = 0.22$ respectively) between the three smoking categories, namely non-smokers, current smokers and former smokers. Smoking and age were therefore not adjusted for. While it is known that gender influences both PAI-1 and fibrinogen levels, the two-way ANCOVA indicated for both PAI-1 and fibrinogen that gender did not significantly affect the association between alcohol consumption and these two factors and therefore it was not considered necessary to adjust or stratify for gender in further analyses investigating these associations.

To determine further possible confounders of fibrinogen and PAI-1 to adjust for, correlations were used. High sensitivity C-reactive protein (CRP) correlated most strongly with fibrinogen (r = 0.42). In the case of PAI-1_{act} the strongest correlations were found with triglycerides (r = 0.29) and waist circumference (r = 0.36). Because of the significance of these correlations we adjusted for CRP in the case of fibrinogen and for triglycerides and waist circumference for PAI-1 in all further analyses.

4.3. POSSIBLE USE OF GGT AND %CDT AS BIOMARKERS FOR ALCOHOL CONSUMPTION

Because self-reported alcohol consumption data are not always reliable, especially in cases where alcohol is abused, we wanted to investigate whether GGT and %CDT could be used as proxy markers for alcohol consumption in this population. The correlations between GGT and alcohol consumption (r = 0.24), and %CDT and alcohol consumption (r = 0.15) in drinkers were, however, very weak. For this reason, only the reported amounts of alcohol consumed were used in this study to determine the association between alcohol consumption and PAI-1_{act} and fibrinogen.

4.4. SUBJECT CHARACTERISTICS

The baseline characteristics of the study participants are presented in Table 4.1. Of the 2010 black African subjects (mean age 48.3 years) who participated in this South African arm of the PURE study, 37.3% were men and 62.7% women. Mean blood pressure values were within the normal range for adults (SBP 131 mmHg, DBP 86.5 mmHg). The geometric means of GGT, 56.2 U/l (53.9-58.4 U/l) and %CDT, 2.66% (2.60-2.72%) fell within the normal ranges. The reference range for CRP in healthy population groups are between 0 to 3 mg/l, and the geometric mean CRP concentration for this population was 3.14 mg/l (2.93-3.37 mg/l). Fibrinogen concentrations and PAI-1_{act} levels of the total population of 3.16 g/l (3.08-3.25 g/l) and 3.55 IU/ml (3.36-3.75

IU/ml) respectively, were within the reference ranges for healthy Caucasian individuals. These reference ranges were used because the ranges for black Africans are still unknown. Mean triglyceride concentrations of participants were 1.14 mmol/l (1.11-1.16 mmol/l), which are below the cut-off value for increased triglycerides of 1.69 mmol/l, indicating normal mean triglyceride concentrations. Of this population, 2.62% of participants were homozygous for the 4G allele, 24.8% presented with the 4G/5G genotype, and the majority of the population (72.5%) was homozygous for the 5G allele. Fifty-two percent of the population were smokers and 16.2% were HIV positive. The subjects recruited to take part in this study were apparently healthy, and consequently the participants in the cohort who were HIV positive did not know their HIV status prior to the commencement of the study and did not use antiretroviral therapy. The amount of alcohol consumed by the study participants who consumed alcohol was approximately 13.9 (13.8-16.2) gram per day. Men consumed significantly higher amounts (17.2 g (16.5-20.1 g)) of alcohol than women (10.9 g (10.7-13.6 g)). However, no difference was seen in the amounts of alcohol consumed by rural and urban groups.

Table 4.1: Baseline characteristics

Variable	PURE study population Geometric mean (95% CI)
Total n	2010
Urban f (%)	1004 (50.0%)
Rural f (%)	1006 (50.0%)
Men/women f (%)	749 (37.3%)/1261 (62.7%)
Age (yr)	48.3 (47.9-48.7)
SBP (mmHg)	131 (130-132)
DBP (mmHg)	86.5 (85.9-87.2)
Waist circumference (cm)	78.7 (78.2-79.3)
GGT (U/l) (▼80U/l for men, 50U/l for women)	56.2 (53.9-58.4)
%CDT (%) (▼2.6%)	2.66 (2.60-2.72)
Triglycerides (mmol/L) (▲0<1.69 mmol/L)	1.14 (1.11-1.16)
Fibrinogen concentration (g/L) (•2.43-3.23 g/L)	3.16 (3.08-3.25)
CRP (mg/L) (▲0-3 mg/L)	3.14 (2.93-3.37)
PAI-1_{act} (IU/ml) (•5.5-10 IU/ml)	3.55 (3.36-3.75)
4G/4G f (%)	47 (2.62%)
4G/5G f (%)	446 (24.8%)
5G/5G f (%)	1302 (72.5%)
Smoking	
Current f (%)	1042 (52.1%)
Never f (%)	881 (44.1%)
Former f (%)	77 (3.9%)
HIV+ f (%)	326 (16.2%)
Alcohol consumption (only drinkers) (f = 1949)	
Total group (g/day)	13.9 (13.8-16.2)
Women (g/day)	10.9 (10.7-13.6) *
Men (g/day)	17.2 (16.5-20.1) *
Rural (g/day)	13.7 (13.1-17.5)
Urban (g/day)	14.0 (13.7-16.4)

SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI body mass index; CRP:C-reactive protein; GGT: gamma-glutamyl transferase; %CDT: percentage carbohydrate deficient transferrin; HIV: human immunodeficiency virus; f: frequency

▼ *Cut-off values recommended by manufacturers of test procedures*

•*Reference values given for a healthy population by the manufacturers of the test procedure*

▲ *Reference ranges in healthy population groups (Baron, 2004)*

* *Means with the same symbol differed significantly for alcohol consumption (g/day)*

4.5. POPULATION CHARACTERISTICS OF DIFFERENT DRINKING CATEGORIES

In order to determine population characteristics of study participants who consume different amounts of alcohol, the study population was divided into drinking categories. Participants who reported consuming no alcohol were classified as non-drinkers. Men who consumed less than 30 g (approximately 2 units per day) and women who consumed less than 15 g (approximately 1 unit per day) alcohol per day, were classified as moderate drinkers. The category of heavy drinkers was constituted of women who drank 15 g alcohol and more per day, and men who drank 30 g alcohol and more per day. In Table 4.2 various population characteristics of the non-drinkers, moderate drinkers and heavy drinkers are shown. The majority (55.3%) of the PURE population were non-drinkers, while 26.6% were moderate and 18.2% heavy drinkers. The Pearson Chi-square test indicated a significant association between alcohol consumption and gender ($p < 0.0001$), this means that there was a difference in alcohol consumption between men and women. Thirty-five percent of the men and 66.9% of the women who participated in this study were non-drinkers. There was a larger percentage (41.7%) of men than of women (17.6%) who were moderate drinkers. Twenty-two percent of men were heavy drinkers, while 15.5% of women drank heavily. No age differences were observed between non-drinkers, moderate drinkers and heavy drinkers ($p = 0.06$). Before adjustment for triglycerides and waist circumference, which were shown to be confounders for PAI-1_{act}, no differences in PAI-1_{act} were seen between drinking categories ($p = 0.14$). When PAI-1_{act} was adjusted for triglycerides and waist circumference, the level of PAI-1_{act} was significantly increased with increased alcohol consumption ($p = 0.036$). It was also observed that the mean waist circumferences of non-drinkers (80.5 cm) were significantly higher than those of the moderate (76.5 cm) and heavy (76.5cm) drinkers ($p < 0.0001$). No differences were observed for triglyceride concentrations between the non-drinkers (1.15 mmol/l), moderate drinkers (1.12 mmol/l) and heavy drinkers (1.12 mmol/l). The fibrinogen concentrations of the non-drinkers were significantly higher than those of both moderate and heavy drinkers, while no differences were seen between moderate and heavy

drinkers. The BMI of non-drinkers (25.3 kg/m^2) was also significantly higher ($p < 0.0001$) than that of the moderate (22.0 kg/m^2) and heavy drinkers (21.8 kg/m^2). A significant difference in alcohol consumption was observed for the different smoking categories (Pearson Chi-square test $p < 0.0001$). Fifty-seven percent of non-drinkers were also non-smokers, but only 28.0% of moderate drinkers, and 25.3% of heavy drinkers were non-smokers. Forty percent of current smokers did not drink alcohol, 35.0% drank moderately, and 24.5% of current smokers were also heavy drinkers. But of those who consumed alcohol heavily, 70.1% were also current smokers.

Table 4.2: Geometric mean (95%CI) of population characteristics for non-drinkers, moderate drinkers and heavy drinkers

Variable	Non-drinkers	Moderate drinkers•	Heavy drinkers†	p-value
Total f (%)	1077 (55.3%)	518 (26.6%)	354 (18.2%)	
Men f(%)	259 (35.7%)	303 (41.7%)	164 (22.6%)	p < 0.0001
Women f (%)	818 (66.8%)	215 (17.6%)	190 (15.5%)	
Age (years)	48.5 (48.1-49.4) [f=1077]	48.3 (47.5-49.1) [f=518]	47.3 (46.5-48.2) [f=354]	0.06
PAI-1_{act} (U/ml) unadjusted	3.69 (3.43-3.95) [f=1023]	3.30 (2.95-3.69) [f=498]	3.86 (3.34-4.43) [f=336]	0.14
PAI-1_{act} (U/ml) adjusted for triglycerides and waist circumference	3.47 (3.23-3.72) [f=1023]	3.57 (3.16-3.88) [f=498]	4.14 (3.68-4.65) [f=336]	0.036
Fibrinogen concentration (g/l)	3.36 (3.25-3.48)* [f=946]	2.95 (2.81-3.11) [f=464]	2.89 (2.72-3.08) [f=316]	<0.0001•
Waist circumference (cm)	80.5 (79.7-81.3)* [f=1071]	76.5 (75.5-77.4) [f=510]	76.5 (75.3-77.6) [f=348]	<0.0001
BMI (kg/m^2)	25.3 (24.9-25.7)* [f=1030]	22.0 (21.5-22.4) [f=481]	21.8 (21.3-22.4) [f=325]	<0.0001
Triglycerides (mmol/l)	1.15 (1.12-1.18) [f=1009]	1.12 (1.07-1.17) [f=477]	1.12 (1.07-1.19) [f=338]	0.61
Smoking				
Non-smokers f (%)	615 (72.4%)	145 (17.1%)	89 (10.5%)	
Previous f (%)	46 (60.5%)	15 (19.7%)	15 (19.7%)	p < 0.0001
Current f (%)	411 (40.5%)	355 (35.0%)	248 (24.5%)	

•Moderate drinking: >0<15 g/day for women (1 unit/day); >0<30 g/day for men (2 units/day)
†Heavy drinking: ≥15 g/day for women (>1 unit/day); ≥30 g/day for men (>2 units/day)
**Mean differed significantly from other drinking categories*
•P-value reported for fibrinogen concentration adjusted for CRP
f: frequency
BMI: Body mass index
%: Percentage of participants in drinking categories for each specific characteristic, e.g. gender, smoking

4.6. PAI-1_{ACT} IN POPULATION SUB-DIVISIONS AND ASSOCIATION WITH ALCOHOL CONSUMPTION

The influence of various population sub-divisions on the association between alcohol consumption and PAI-1_{act} is reported in Table 4.3. Women (4.56 IU/ml) had significantly higher PAI-1_{act} than men (2.29 IU/ml). Non-drinking women had significantly lower PAI-1_{act} than women who were heavy drinkers (4.30 IU/ml *v.* 5.70 IU/ml), whereas the PAI-1_{act} of moderate and heavy drinkers (women) was not significantly different. PAI-1_{act} of men tended to increase with increased alcohol consumption ($p = 0.06$). The association between alcohol consumption and PAI-1_{act} was not significantly influenced by gender ($p = 0.67$).

Urban participants had significantly higher PAI-1_{act} than rural participants (4.26 and 2.96 IU/ml respectively). PAI-1_{act} decreased significantly with moderate drinking ($p = 0.002$), but then increased again significantly with heavy alcohol consumption in the urban participants ($p = 0.01$). No association was found between alcohol consumption and PAI-1_{act} in the rural group. Despite the significant decrease with moderate consumption and increase with heavy alcohol consumption in urban participants, the two-way ANCOVA indicated that urbanisation did not significantly influence the association between PAI-1_{act} and alcohol consumption ($p = 0.16$).

In the 4G/5G polymorphism sub-division, the PAI-1_{act} of participants who were homozygous for the 4G allele had significantly higher PAI-1_{act} than participants who were homozygous for the 5G allele (5.87 *v.* 3.35 IU/ml), with participants who were heterozygous (4G/5G) presenting with intermediate PAI-1_{act} levels of 3.90 IU/ml (3.48-4.37IU/ml). When comparing PAI-1_{act} between drinking categories in each of the 4G/5G polymorphism sub-divisions, no association between alcohol and PAI-1_{act} was observed, nor did the 4G/5G polymorphism influence the association between alcohol consumption and PAI-1_{act} significantly ($p = 0.46$). It should however, be noted that dividing the participants with the 4G/4G genotype into drinking categories, resulted in relatively small numbers per group (24, 12 and 10, respectively). The possibility can therefore not be excluded that we may have been under powered in the comparison of PAI-1_{act} levels between the drinking categories in the 4G/4G genotype participants.

Next, study participants were categorised, on the basis of waist circumference, as abdominally obese or as having normal waist circumference measures (≤ 88 cm for women, ≤ 102 cm for men). The abdominally obese participants had significantly higher PAI-1_{act} levels (6.78 IU/ml) than participants with normal waist circumference measurements (2.98 IU/ml). In the abdominally obese group, PAI-1_{act} increased with increased alcohol consumption; this increase was significant with heavy alcohol consumption ($p = 0.03$). Before adjustment for triglycerides, waist circumference had a significant influence on the association between alcohol consumption and PAI-1_{act} ($p = 0.016$); however, after adjustment, the two-way ANCOVA indicated no significance.

Participants with normal triglyceride concentrations (<1.7 mmol/l) had significantly lower PAI-1_{act} (3.01 IU/ml) than those with high triglyceride concentrations (6.52 IU/ml). A significant decrease in PAI-1_{act} was seen between non-drinkers and participants who consumed moderate amounts of alcohol and had normal triglyceride concentrations, but not in participants with increased triglyceride concentrations. In participants with normal triglyceride concentrations, PAI-1_{act} did not decrease further with consumption of larger amounts of alcohol. In the group with abnormal triglyceride concentrations, PAI-1_{act} increased with increased alcohol consumption ($p = 0.007$). The two-way ANCOVA indicated that the influence of triglycerides on alcohol consumption and PAI-1_{act} was significant ($p = 0.011$).

In the BMI sub-division, a significant increase in PAI-1_{act} levels was seen with increasing BMI, except in the overweight (BMI = 25-29.9 kg/m^2) and obese (BMI ≥30 kg/m^2) categories. PAI-1_{act} tended to increase with alcohol consumption in both normal ($p = 0.06$) and overweight volunteers, but this increase reached significance only in the obese individuals ($p = 0.056$). However, BMI did not influence the association between alcohol consumption and PAI-1_{act} significantly, based on the two-way ANCOVA ($p = 0.42$).

Table 4.3: The influence of various population sub-divisions on the association of alcohol consumption with PAI-1_{act}

Sub-divisions	Total group Geometric mean (95%CI) [f]	Non-drinkers Geometric mean (95%CI) [f]	Moderate drinkers• Geometric mean (95%CI) [f]	Heavy drinkers† Geometric mean (95%CI) [f]	ANCOVA between drinking categories p-value	Two-way ANCOVA Interaction p-value
Women	[a]4.56 (4.29-4.85) [f=1188]	4.30 (3.99-4.63)* [f=770]	4.97 (4.26-5.77) [f=203]	5.70 (4.86-6.67)* [f=180]	0.00019	0.67
Men	[a]2.29 (2.06-2.53) [f=725]	2.22 (1.87-2.62) [f=253]	2.44 (2.08-2.84) [f=295]	2.35 (1.83-2.96) [f=156]	0.06	
Urban	[b]4.26 (3.94-4.59) [f=941]	4.66 (4.21-5.14)* [f=423]	3.57 (3.08-4.12)*✦ [f=303]	4.69 (3.92-5.59)✦ [f=197]	0.017	0.16
Rural	[b]2.96 (2.74-3.20) [f=972]	3.10 (2.82-3.41) [f=600]	2.93 (2.44-3.49) [f=195]	2.88 (2.27-3.60) [f=139]	0.83	
4G/4G	[c]5.87 (4.10-8.25) [f=47]	5.11 (3.16-7.98) [f=24]	8.67 (4.92-14.8) [f=12]	6.30 (1.80-18.0) [f=10]	0.77	
4G/5G	3.90 (3.48-4.37) [f=446]	4.27 (3.68-4.93) [f=235]	3.45 (2.73-4.32) [f=129]	4.10 (2.91-5.66) [f=67]	0.30	0.46
5G/5G	[c]3.35 (3.13-3.58) [f=1302]	3.38 (3.10-3.68) [f=694]	3.12 (2.71-3.58) [f=331]	3.83 (3.24-4.50) [f=238]	0.02	
Normal WC	[d]2.98 (2.79-3.17) [f=1512]	3.06 (2.81-3.32) [f=756]	2.84 (2.51-3.20) [f=431]	3.27 (2.78-3.82) [f=282]	0.22	0.11
Abdominal obesity	[d]6.78 (6.15-7.47) [f=383]	6.15 (5.48-6.90)* [f=262]	8.20 (6.35-10.5) [f=60]	9.75 (7.60-12.5)* [f=48]	0.018	(0.016)◊
Triglycerides						
<1.7mmol/l	[e]3.01 (2.83-3.20) [f=1497]	3.24 (2.99-3.51)* [f=799]	2.65 (2.33-3.11)* [f=386]	3.17 (2.68-3.72) [f=267]	0.11	0.011
≥1.7mmol/l	[e]6.52 (5.82-7.29) [f=367]	5.84 (5.01-6.79) [f=200]	7.53 (5.93-9.51) [f=89]	7.88 (6.14-10.0) [f=67]	0.007	
BMI						
<18.5	1.81 (1.54-2.11) [f=330]	1.96 (1.53-2.46) [f=122]	1.56 (1.16-2.04) [f=113]	2.03 (1.42-2.80) [f=79]	0.32	0.42
18.5-24.9	2.87 (2.63-3.13) [f=788]	2.69 (2.37-3.04) [f=372]	3.01 (2.57-3.50) [f=237]	3.44 (2.78-4.21) [f=162]	0.06	
25-29.9	[1]5.13 (4.59-5.71) [f=321]	4.73 (4.18-5.34) [f=221]	6.22 (4.64-8.25) [f=58]	7.30 (4.90-10.7) [f=31]	0.11	
≥30	[1]6.12 (5.51-6.79) [f=370]	5.63 (4.97-6.36)* [f=266]	7.39 (5.65-9.58) [f=55]	9.01 (6.66-12.1)* [f=38]	0.056	

WC: waist circumference; BMI: body mass index; abdominal obesity: WC >88 cm in women and >102 cm in men
•Moderate drinking: >0<15 g/day for women (1 unit/day); >0<30 g/day for men (2 units/day)
†Heavy drinking: ≥15 g/day for women (>1 unit/day); ≥30 g/day for men (>2 units/day)
**✦Means with the same symbol differed significantly between alcohol consumption categories*
a, b, c, d, e: Means with the same symbol differed significantly within sub-divisions in the total group
1: Only BMI categories that did not differ significantly from each other in the total group
f: frequency
ANCOVA adjusted for triglycerides and WC, except in the case of triglycerides (adjusted only for WC) and WC and BMI (adjusted orily for triglycerides)
◊: Before adjustment for triglycerides

4.7. FIBRINOGEN CONCENTRATIONS IN POPULATION SUB-DIVISIONS AND ASSOCIATION WITH ALCOHOL CONSUMPTION

In Table 4.4 the influence of various population sub-divisions on the association between alcohol consumption and fibrinogen concentration is presented.

Women had significantly higher fibrinogen concentrations (3.36 g/l) than men (2.86 g/l). In women a negative correlation was seen with alcohol consumption ($p = 0.0019$). In men fibrinogen decreased with moderate alcohol consumption, but did not decrease further with heavy consumption. The two-way ANCOVA did not indicate a significant influence of gender on the association between alcohol consumption and fibrinogen concentration ($p = 0.31$).

Significantly higher fibrinogen concentrations were observed in rural participants (3.32 g/l) than in urban participants (3.00 g/l). Fibrinogen concentrations decreased significantly with heavy alcohol consumption in the rural sub-division ($p = 0.004$). In the urban participants, fibrinogen concentrations decreased significantly with moderate alcohol consumption ($p = 0.0013$); however, drinking higher amounts of alcohol did not lead to further reductions in fibrinogen concentrations. Living in either a rural or an urban area does not seem to influence the association between alcohol consumption and fibrinogen concentrations significantly ($p = 0.37$).

Abdominally obese participants (waist circumference greater than 88 cm for women and 102 cm for men) had significantly higher fibrinogen concentrations (3.71 g/l) than participants with normal waist circumference (3.04 g/l). Significantly reduced fibrinogen concentrations ($p < 0.0001$) were seen with moderate alcohol consumption in participants with normal waist circumference. Consuming higher amounts of alcohol did not lead to a further decrease in fibrinogen concentrations in these participants. In abdominally obese participants the consumption of alcohol was not associated with reduced fibrinogen concentrations. The two-way ANCOVA did not, however, indicate

that waist circumference had a significant influence on the association between alcohol consumption and fibrinogen concentrations.

Significantly higher fibrinogen concentrations were seen in obese study participants when compared with participants in other categories in the BMI sub-division. In participants with normal BMI (BMI = 18.5-24.9 kg/m^2) and those who were overweight (BMI = 25-29.9 kg/m^2), moderate alcohol consumption led to decreased fibrinogen concentrations, while consuming higher amounts of alcohol did not further decrease fibrinogen concentrations. Alcohol consumption did not, however, lead to decreased fibrinogen concentration in underweight (BMI <18.5 kg/m^2) and obese (BMI ≥30 kg/m^2) participants. BMI did not seem to influence the association between alcohol consumption and fibrinogen concentration significantly ($p = 0.43$). Fibrinogen strongly correlated with CRP, and therefore fibrinogen was adjusted for CRP in all analyses.

Table 4.4: The influence of various population sub-divisions on the association of alcohol consumption with fibrinogen concentration

Sub-divisions	Total group Geometric mean (95%CI) [f]	Non-drinkers Geometric mean (95%CI) [f]	Moderate drinkers• Geometric mean (95%CI) [f]	Heavy drinkers† Geometric mean (95%CI) [f]	ANCOVA p-value between drinking categories	Two-way ANCOVA Interaction p-value
Women	[a]3.36 (3.25-3.48) [f=1109]	3.48 (3.34-3.63)* [f=714]	3.26 (3.02-3.52) [f=195]	3.02 (2.76-3.30) * [f=165]	0.0019	0.31
Men	[a]2.86 (2.74-2.98) [f=673]	3.03 (2.84-3.23) [f=232]	2.75 (2.57-2.94) [f=269]	2.76 (2.53-3.01) [f=151]	0.012	
Rural	[b]3.32 (3.20-3.44) [f=932]	3.45 (3.30-3.61) * [f=567]	3.14 (2.89-3.41) [f=191]	2.98 (2.71-3.28) * [f=136]	0.004	0.37
Urban	[b]3.00 (2.89-3.12) [f=850]	3.23 (3.06-3.42)*• [f=379]	2.83 (2.65-3.01)• [f=273]	2.83 (2.61-3.07) * [f=180]	0.0013	
Normal WC	[c]3.04 (2.96-3.13) [f=1414]	3.22 (3.09-3.35)*• [f=699]	2.87 (2.72-3.03)• [f=404]	2.85 (2.67-3.04) * [f=268]	<0.0001	0.24
Abdominal obesity	[c]3.71 (3.50-3.93) [f=352]	3.79 (3.54-4.07) [f=242]	3.75 (3.22-4.37) [f=55]	3.24 (2.68-3.92) [f=42]	0.14	
BMI						
<18.5	3.19 (3.00-3.39) [f=306]	3.36 (3.04-3.71) [f=109]	2.99 (2.69-3.33) [f=105]	3.19 (2.84-3.59) [f=76]	0.085	0.43
18.5-24.9	2.89 (2.78-3.01) [f=741]	3.08 (2.91-3.26)*• [f=348]	2.74 (2.54-2.95) • [f=223]	2.73 (2.49-2.99)* [f=153]	0.0013	
25-29.9	3.18 (2.99-3.38) [f=302]	3.27 (3.04-3.53) [f=209]	2.90 (2.50-3.36) [f=53]	2.91 (2.40-3.53) [f=29]	0.044	
≥30	[2]3.90 (3.68-4.14) [f=338]	3.95 (3.69-4.23) [f=244]	4.00 (3.47-4.63) [f=51]	3.41 (2.72-4.27) [f=32]	0.3	

WC: waist circumference; BMI: body mass index; abdominal obesity: WC >88 cm in women and >102 cm in men
•Moderate drinking: >0<15 g/day for women (1 unit/day); >0<30 g/day for men (2 units/day)
†Heavy drinking: ≥15 g/day for women (>1 unit/day); ≥30 g/day for men (>2 units/day)
***Means with the same symbol differed significantly between alcohol consumption categories*
a, b, c: Means with the same symbol differed significantly within sub-divisions in the total group
2: Mean differed significantly from other BMI divisions in the BMI sub-division
f: frequency
ANCOVA adjusted for CRP

4.8. MULTIPLE REGRESSION TO DETERMINE POSSIBLE PREDICTORS OF VARIANCE IN FIBRINOGEN CONCENTRATION AND PAI-1_{ACT}

In order to determine possible predictors of fibrinogen concentration in this study population, a multiple regression model was used. The multiple regression model in which the rural and urban sub-division, gender, alcohol consumption and CRP were included indicated CRP to be the main predictor of the variance in fibrinogen concentrations, explaining 17% of the variance. Only 1% of the variance in fibrinogen concentrations could be explained by alcohol consumption.

A multiple regression model was also used to determine possible predictors for PAI-1_{act}. In a model which included waist circumference, triglycerides, gender, alcohol consumption, the 4G/5G polymorphism and living in a rural or urban area, waist circumference and triglyceride concentration were shown to be responsible for 12% and 5% of the variance in PAI-1_{act} respectively, whereas gender explained 3%, and alcohol consumption 1% only.

CHAPTER 5: DISCUSSION AND CONCLUSION

5.1. INTRODUCTION

No information is available regarding the two haemostatic risk factors, PAI-1_{act} and fibrinogen, and their association with alcohol consumption in black South Africans. We wanted to investigate this association, if any, between habitual alcohol consumption, PAI-1_{act} and fibrinogen levels in a black South African population, and also how sub-divisions such as gender, urbanisation, waist circumference and BMI, as well as, in the case of PAI-1, how triglyceride concentrations and the 4G/5G polymorphism, influence the association between alcohol consumption, PAI-1_{act} and fibrinogen in this population.

These sub-divisions were chosen because they have themselves been shown to influence PAI-1_{act} and fibrinogen concentrations. Although this is not the main focus of this dissertation, the influence of these factors on PAI-1_{act} and fibrinogen is discussed in sections 5.2 and 5.3, after which the association between alcohol consumption, PAI-1_{act} and fibrinogen, and the influence of the above-mentioned factors, if any, on these associations is dealt with in sections 5.4 and 5.5, followed by a review of the limitations of this study, the conclusion, and recommendations for future research.

5.2. THE INFLUENCE OF VARIOUS POPULATION SUB-DIVISIONS ON PAI-1_{ACT}

The following section deals with the influence of gender, urbanisation, the 4G/5G polymorphism, waist circumference, triglyceride concentration and BMI on PAI-1_{act}.

5.2.1. The influence of gender on PAI-1_{act}

In the South African PURE population, women had significantly higher PAI-1_{act} than men. Various studies in Caucasian populations have, however, shown men to have higher PAI-1 than women (Krishnamurti *et al.*, 1988; Lacroix *et al.,* 1996; Chadarevian

et al., 1999; Van Harmelen *et al.*, 2000), while Stegnar & Pentek (1993) and Wimen *et al.* (2000) found no difference in PAI-1 between healthy Caucasian men and women. In a black South African population Nienaber *et al.* (2008) also observed higher PAI-1_{act} in girls than in boys. This was attributed partly to the girls having higher fat percentages than the boys; however, when the researchers adjusted for body fat percentage and physical activity, the levels of PAI-1_{act} in the girls remained significantly higher than in the boys. Thus the reason for the potentially higher levels of PAI-1_{act} in black African women than in men seems to be gender-related but is unlikely to be only the result of gender-related differences in body composition.

5.2.2. The influence of urbanisation on PAI-1_{act}

The urban participants in the PURE population presented with significantly higher PAI-1_{act} than the rural participants. In other studies in which black South Africans participated, Jerling *et al.* (1994) found rural men to have very low PAI-1_{act} when compared with studies involving participants in transition (Pieters *et al.*, 2006; Nienaber *et al.*, 2008) or living in urban areas (Greyling *et al.*, 2006). Thus, even though the number of studies investigating PAI-1_{act} in black South Africans in different stages of transition is limited, PAI-1_{act} seems to increase with urbanisation. The increased PAI-1_{act} seen with urbanisation may be related to the increased prevalence of non-communicable diseases associated with urbanisation or to dietary changes that cause a worsening of the lipid profile. These include decreased intake of plant protein and a decrease in the polyunsaturated/saturated fat ratio and carbohydrates as well as increased intake of animal protein and total fat (James *et al.*, 2000). There does, however, seem to be a genetic protective influence on PAI-1 in black African populations, as will be discussed in the following section.

5.2.3. PAI-1_{act} levels and the possible role of the 4G/5G polymorphism on PAI-1_{act} levels

The geometric mean PAI-1_{act} values for the total PURE population was 3.55 (3.36-3.75) IU/ml. Results of the present study are consistent with data from other studies in which black Africans participated (Jerling *et al.*, 1994; Greyling *et al.*, 2006) and also with

those of African-Americans (Festa *et al.*, 2003). The reference ranges given for a healthy Caucasian population by the manufacturers of the test kit used to measure the PAI-1_{act} was 5.5 ± 5 IU/ml. Thus, the geometric mean PAI-1_{act} values of the PURE population fell well within the lower end of this given reference range. Greyling *et al.* (2006) and Jerling *et al.* (1994) also found that Africans present with significantly lower PAI-1_{act} in comparison with Caucasians. We suggest that, because of the apparent consistent differences in PAI-1 levels between black Africans and Caucasians, ethnic-specific healthy ranges should be developed. Genetic influences, specifically the 4G/5G polymorphism, have been investigated as a possible explanation for the lower PAI-1 levels observed in black South Africans. The 4G/5G polymorphism has been linked to differences in PAI-1 levels, because the 5G allele binds not only a transcriptional activator but also a repressor protein to an overlapping binding site, as explained in section 2.7 of Chapter 2.

We observed the highest PAI-1_{act} levels in participants homozygous for the 4G allele, intermediate PAI-1_{act} in 4G/5G participants and the lowest PAI-1_{act} in participants homozygous for the 5G allele, which is consistent with what is found in Caucasian populations (Margaglione *et al.*, 1997; Burzotta *et al.*, 1998; Sartori *et al.*, 2001; Festa *et al.*, 2003). In the present study, 72.5% of the population was homozygous for the 5G allele and only 2.62% for the 4G allele. Naran *et al.* (2008) found the 4G allelic frequency to be much higher in a Caucasian population than in a black South African population. The percentage of black African participants in their study who were homozygous for the 4G/4G genotype was only 2.8% (comparable with that found in our study) as opposed to 36.4% in white participants. In the study of Naran *et al.* (2008), 77.6% of black Africans and 19.3% of white participants presented with the 5G/5G genotype. These high frequencies (72.5 and 77.6%) of the 5G/5G genotype in black African populations might therefore explain the lower PAI-1_{act} levels observed in these populations when compared with data from studies on Caucasian participants.

5.2.4. The influence of waist circumference and BMI on PAI-1_{act}

In the South African PURE population, participants with abdominal obesity had significantly higher PAI-1_{act} than participants with normal waist circumference measurements. We also found that participants who were overweight or obese had significantly higher PAI-1_{act} than participants within the normal or underweight BMI ranges. Thus PAI-1_{act} increased with increasing BMI and waist circumference. PAI-1 is synthesised by various sources and primary sources have been suggested to be the hepatocytes, adipocytes, platelets, endothelial cells and VSMC, but uncertainty still exists regarding which of these sources is the predominant origin of circulating PAI-1 under normal and different pathological conditions (Juhan-Vague *et al.*, 2003; Dellas & Loskutoff, 2005). However, in individuals who are overweight or obese, and specifically in individuals who present with an abdominal pattern of fat distribution, adipose tissue, especially visceral adipose tissue, has been shown to be an important source of PAI-1 (Alessi *et al.*, 1997; Juhan-Vague *et al.*, 2002; Skurk & Hauner, 2004). In fact, Bastelica *et al.* (2002) found that visceral fat produces five times more PAI-1 than does subcutaneous fat. This may be attributed to stromal cells, which it has been suggested are the main PAI-1 producing cells, and are more numerous in visceral fat (Bastelica *et al.*, 2002). This is a likely explanation for why PAI-1 was significantly increased in individuals with abdominal obesity and why waist circumference is considered to be such an important contributor to plasma PAI-1 levels. It is likely, therefore, that the higher PAI-1_{act} in participants within the higher BMI ranges (regardless of fat distribution) is also due to the increased PAI-1 production by adipose tissue in general. Although subcutaneous fat is not the main source of PAI-1 (compared with visceral fat), the higher percentage of total fat in participants within the higher BMI ranges would still produce more PAI-1 than is seen in leaner individuals. Participants who present with overweight or obesity also have a higher likelihood of being centrally obese, compared with lean/ normal weight individuals.

5.2.5. The influence of triglyceride concentration on PAI-1_{act}

In the present study, participants with increased triglyceride concentrations (> 1.7 mmol/l) had significantly higher PAI-1_{act} levels than participants with normal triglyceride

concentrations. Increased triglyceride levels have also been associated with increased PAI-1 levels in other studies (Delahouse *et al.*, 2001; Krebs *et al.*, 2003; Peverill *et al.*, 2007). Chadarevian *et al.* (1999) found the relationship between PAI-1 and triglycerides to be linear and continuous, without a threshold value. A strong relationship between VLDL and PAI-1 has also been observed (Nilsson *et al.*, 1999). Various *in vitro* studies have investigated the mechanism by which very-low-density lipoprotein (VLDL), composed primarily of triglycerides, induced increased PAI-1 transcription and secretion by endothelial cells (Stiko-Rahm *et al.*, 1990; Nilsson *et al*, 1998; Nilsson *et al.,* 1999). Although it is not yet exactly clear how VLDL triglycerides induce increased PAI-1, a VLDL response element by which VLDL induces PAI-1 expression was identified by Eriksson *et al.* (1998) in the promoter region of the PAI-1 gene. Also, a VLDL receptor has been found on endothelial cells (Nilsson *et al.,* 1999), and Stiko-Rahm *et al.* (1990) found a direct relation between the amount of VLDL bound to these B and E receptors on endothelial cells, and the amount of PAI-1 which is secreted by these cells. It was also found that endothelial cells bound hypertriglyceridaemic VLDL more effectively than normotriglyceridaemic VLDL (Stiko-Rahm *et al.*, 1990), which may be why we found participants with increased triglycerides to have higher $PAI\text{-}1_{act}$ in the present study.

5.3. THE INFLUENCE OF VARIOUS POPULATION SUB-DIVISIONS ON FIBRINOGEN CONCENTRATION

In the following section, the influence of gender, urbanisation, waist circumference and BMI on fibrinogen concentration is discussed.

5.3.1. The influence of gender on fibrinogen concentration

In the present study, women presented with significantly higher fibrinogen concentrations than men. Vorster *et al.* (1998) also found women to have higher fibrinogen concentrations than men in black African populations, and Nienaber *et al.* (2008) found higher fibrinogen concentrations in African girls than in African boys;

however, this difference was not significant. Results from the THUSA study (Transition and Health during Urbanisation of South Africans), which ten years ago studied black South Africans in the North West region from communities similar to those in the PURE study, also showed that women presented with higher fibrinogen concentrations than men (James *et al.*, 2000). In a Caucasian population, women were also found to also have higher fibrinogen concentrations than men (Mennen *et al.*, 1999), while Rana *et al.* (2009) found no differences between the fibrinogen concentrations of healthy Caucasian men and women. A possible reason for the higher fibrinogen concentrations in women may be the higher fat percentages, which are associated with sub-clinical inflammation, as adipose tissue is considered to be inflammatory active tissue (as will be discussed in section 5.3.2). Fibrinogen is an acute-phase protein, the concentration of which is increased in inflammation (Gabay & Kushner, 1999). Fibrinogen also strongly correlates with CRP, an acute-phase protein generally used as a proxy marker for inflammation (Gabay & Kushner, 1999). In the PURE study too, fibrinogen correlated strongly with CRP ($r = 0.42$; $p < 0.0001$). Interestingly enough, fibrinogen was higher in women than in men, even after adjustment for CRP. This suggests that fibrinogen is increased in women not only via an inflammatory-related adipose tissue link but also through other as yet unknown mechanisms.

5.3.2. The influence of waist circumference and BMI on fibrinogen concentration

Participants who presented with abdominal obesity had significantly higher fibrinogen concentrations than participants with normal waist circumference measurements. The fibrinogen concentrations of participants who were obese were also significantly higher than those of participants in the lower BMI ranges. Nguyen *et al.* (2009) found increased fibrinogen concentrations across weight classes where increases of 0.115 g/l for overweight and 0.256 g/l for obese participants were seen when comparing fibrinogen concentration with that of individuals of normal weight participating in their study. As mentioned in section 5.3.1, obesity is generally considered to be pro-inflammatory. Adipose tissue may be infiltrated by macrophages, or the adipocytes themselves become producers of inflammatory cytokines such as interleukin-1beta (IL-1β), tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) (Yudkin *et al.*, 1999;

Rosenson & Koenig, 2003; Park *et al.*, 2005; Wellen & Hotamisligil, 2005). These cytokines are then transported in the blood to the liver, where increased production of acute-phase proteins, such as fibrinogen is induced (Vasse *et al.*, 1994). Therefore this might explain why participants with higher waist circumference measurements and BMI presented with higher fibrinogen concentrations.

5.3.3. The influence of urbanisation on fibrinogen concentration

In the rural group, participants of the present study had significantly higher fibrinogen concentrations than those in urban areas. CRP was found to correlate strongly with fibrinogen, and was thus adjusted for in all fibrinogen analyses. Therefore inflammation cannot be the only reason for the higher fibrinogen concentration in the rural participants of this study. Rural participants may be at higher risk of inflammatory conditions owing to the fact that unlike urban participants, they may not always have access to clean water and sanitation. James *et al.* (2000), on the other hand, found deep rural participants from the THUSA study to have significantly lower fibrinogen concentrations when compared with those of other urbanisation strata (farms, informal housing or urban African townships and professionals living in urban areas). However, participants who resided on farms, also considered to be rural areas, presented with the highest fibrinogen concentrations across all strata (James *et al.*, 2000). The researchers, James *et al.* (2000) mentioned that these participants who presented with the highest fibrinogen concentrations also had the worst nutritional status among participants from all strata. When comparing the THUSA (data collected 1996-1998) and PURE (data collected 2005) studies, both of which investigated Tswana speaking participants from similar communities, no dramatic difference was observed in the fibrinogen concentration of the urban participants of the two studies, despite participants from the PURE study having been exposed to urbanised living for almost ten years longer. The weighted mean fibrinogen concentration of men (older than 35 years) in THUSA (James *et al.*, 2000) seems slightly higher (3.38 g/l) than the fibrinogen concentration of the men participating in the PURE study (2.86 g/l). Fibrinogen concentrations of the women in THUSA and PURE seem to be in the same range (3.56 g/l and 3.36 g/l respectively). Other studies in which black South Africans participated,

also reported fibrinogen concentrations which seem to be in the same range, irrespective of level of urbanisation (Vorster *et al.*, 1987; Vorster *et al.*, 1998; Nienaber *et al.*, 2008). It therefore seems as if urbanisation *per se* does not influence fibrinogen concentration. When a specific level of urbanisation, however, resulted in a significant worsening of the overall nutritional status, it would then also seem to affect fibrinogen concentration.

5.3.4. The fibrinogen concentrations of PURE study participants, other black Africans and Caucasians

According to the reference values for Caucasian populations given in the test procedure which was used to measure fibrinogen concentrations for the participants of the PURE study, the healthy ranges are between 2.43 – 3.23 g/l. The geometric mean fibrinogen concentration for participants of the South African PURE study was 3.16 (3.08 - 3.25) g/l, which fell in the upper end of the suggested healthy range for Caucasians. This is consistent with fibrinogen concentrations of other black South Africans (Vorster *et al.,* 1987; Jerling *et al.*, 1994; Vorster *et al.,* 1998; James *et al.*, 2000; Greyling *et al.*, 2006; Nienaber *et al.*, 2008). Jerling *et al.* (1994) also found black South African men to have significantly higher fibrinogen concentrations than Caucasian men, and similar results for black women compared with Caucasian women were observed by Greyling *et al.* (2006). Because the reference ranges for Caucasian populations are lower than the fibrinogen concentrations generally observed in black South Africans, we suggest that an ethnic-specific healthy range for fibrinogen concentration be developed for use in the black South African population.

5.4. ASSOCIATION BETWEEN ALCOHOL CONSUMPTION AND PAI-1_{ACT}

No significant differences for unadjusted PAI-1_{act} were seen between drinking groups in the PURE population. However, correlations to determine which confounders for PAI-1_{act} to adjust for, indicated that waist circumference and triglyceride concentration correlate strongly with PAI-1_{act}. After adjustment for triglycerides and waist

circumference, PAI-1_{act} was shown to be significantly increased in the group with heavy alcohol consumption. The participants who did not consume alcohol also had higher waist circumference measurements compared to those who consumed ethanol. This effect of waist circumference on the relationship between alcohol consumption and PAI-1 will be discussed in more detail in section 5.4.1. Epidemiological studies show that PAI-1 also increases in Caucasian populations with heavy alcohol consumption. This occurred typically when more than one standard drink was consumed per day (Marques-Vidal *et al.*, 1995; Djousse *et al.*, 2000; Yarnell *et al.*, 2000; Marques-Vidal *et al.*, 2001; Mukamal *et al.*, 2001; Sasaki *et al.*, 2001). In the present study, heavy alcohol consumption was defined as more than one standard drink per day for women, and more than two standard drinks per day for men. In the South African PURE population no significant increase in PAI-1_{act} was seen with moderate alcohol consumption. In agreement with these results, results from studies in Caucasian participants show that moderate drinking has no influence on PAI-1 (Djousse *et al.,* 2000; Mukamal *et al.*, 2001; Sasaki *et al.*, 2001). As previously mentioned, a significant increase in PAI-1_{act} was observed in the group with heavy alcohol consumption; thus, despite lower PAI-1 values than those of Caucasian populations, the trend in this black African population seems similar to the trends seen in the literature (reported mainly for Caucasians).

The mechanism by which alcohol consumption increases PAI-1_{act} is not yet clear. As mentioned in section 5.2.4, PAI-1 is synthesised by various different sources, thus making it difficult to determine the mechanism/s. A possible mechanism may involve transforming growth factor-β (TGF-β), which is also synthesised in a variety of cells, and is considered to be one of the strongest inducers of PAI-1 synthesis (Juhan-Vague *et al.*, 2002) and which has been shown to be increased following alcohol consumption (Szabo *et al.*, 1992). *In vitro* studies by Ma *et al.* (2002) and Loskutoff *et al.* (1993), in a human trophoblast cell line and in mice respectively, also found that TGF-β increased PAI-1 expression. Another possible mechanism through which PAI-1 is increased with alcohol consumption might involve triglyceride concentration, as PAI-1_{act} of participants with increased triglyceride concentrations increased with alcohol consumption. The

VLDL receptor on endothelial cells and the VLDL response element in the promoter area of the PAI-1 gene, discussed in section 5.2.5, might be some of the pathways through which alcohol affects PAI-1_{act} in participants with increased triglyceride concentrations.

Contrary to these findings, *in vitro* studies investigating the effect of alcohol on endothelial cell gene transcription found that alcohol decreased PAI-1. Booyse *et al.* (1999) and Grenett *et al.* (2000) studied the effect of a low dose of ethanol on transcription of the PAI-1 gene in endothelial cells *in vitro.* The results of these studies indicate a down-regulation in PAI-1 gene expression by ethanol, which may lead to increased fibrinolytic activity *in vivo.* Grenett *et al.* (2000) suggested that the mechanism through which alcohol alters the transcription of PAI-1 possibly involves multiple binding-sites in the promoter and 5'-flanking region of the PAI-1 gene.

It seems, therefore, that the effect of alcohol on PAI-1 is both multiple and complex. Direct effects on different cell types in *in vitro* experiments suggest both decreased and increased PAI-1 responses. PAI-1 is, furthermore, produced by many tissue types which may all be affected differently. Moreover, *in vitro* results cannot be extrapolated to the *in vivo* effects of alcohol on PAI-1, as alcohol can affect PAI-1 *in vivo* not only directly but also indirectly through several pathways. Therefore, while heavy alcohol consumption has consistently been shown to increase PAI-1 in cross-sectional epidemiological studies, the mechanisms behind this effect still need to be determined.

5.4.1. Influence of population sub-divisions on the association of alcohol consumption with PAI-1_{act}

The South African PURE population was divided into sub-divisions of factors which have been shown to influence PAI-1_{act}. Within these sub-divisions two-way ANCOVAs indicated that neither gender nor urbanisation nor the 4G/5G polymorphism nor BMI ranges influenced the association between alcohol consumption and PAI-1_{act}. As mentioned in section 5.2, the highest PAI-1_{act} levels were seen in participants homozygous for the 4G allele, intermediate PAI-1_{act} levels were recorded in 4G/5G

participants, and the lowest levels in participants homozygous for the 5G allele. The 4G/5G polymorphism was specifically investigated in this study because this polymorphism is regarded as a response polymorphism, which means that the differences in PAI-1 levels between the 4G and 5G genotypes become more obvious in the presence of environmental and genetic factors which stimulate the expression of PAI-1 (Hoekstra *et al.*, 2004). However, alcohol does not seem to be a trigger for changes in $PAI\text{-}1_{act}$ levels in the different genotypes.

$PAI\text{-}1_{act}$ levels of participants who were within the normal BMI range tended to increase with increased alcohol consumption, but $PAI\text{-}1_{act}$ was significantly increased with increased alcohol consumption only in obese participants, indicating a possible protective effect against the alcohol-related $PAI\text{-}1_{act}$ increase in individuals with normal weight.

As mentioned in section 5.4, results of the present study indicated that non-drinkers had significantly higher waist circumference measurements than moderate and heavy drinkers. This likely explains why $PAI\text{-}1_{act}$ levels did not differ between drinking and non-drinking participants before adjustment for waist circumference, as higher waist circumferences, are also related to increased $PAI\text{-}1_{act}$. In participants who were abdominally obese (waist circumference > 88cm in women and > 102cm in men), significant increases in $PAI\text{-}1_{act}$ were observed in the group that consumed alcohol heavily. $PAI\text{-}1_{act}$ did not increase, however, with increased alcohol consumption in participants with normal waist circumference measurements. The question that arises is whether having a normal waist circumference will protect against increased $PAI\text{-}1_{act}$ levels associated with alcohol heavy consumption. The two-way ANCOVA indicated that waist circumference had a significant influence on the association between alcohol consumption and $PAI\text{-}1_{act}$. However, this significance disappeared after adjustment for triglycerides, which shows that triglycerides are involved in the mechanism through which central obesity influences participants' $PAI\text{-}1_{act}$ response to alcohol. Furthermore, triglycerides and waist circumference have been shown to correlate strongly (Peverill *et al.*, 2007). Thus the alcohol-related $PAI\text{-}1_{act}$ increase in participants with abdominal

obesity as well as in participants with increased triglyceride concentrations while not observed in individuals with normal waist circumference and triglyceride concentrations, may be an indication of the protective effect of normal waist circumference and a normal triglyceride concentration against this alcohol-related PAI-1_{act} increase.

It is not exactly clear how central obesity and increased triglyceride concentration contribute to increased PAI-1_{act} in heavy alcohol consumption. Possible mechanisms through which alcohol might increase PAI-1 may involve directly increasing PAI-1 production through adipocytes, or through inflammation of adipocytes leading to the production of cytokines and the subsequent production of acute-phase proteins (of which PAI-1 is one). A reason for the increase in PAI-1_{act} with alcohol consumption in participants with increased triglyceride concentrations may be that alcohol affects the involvement or functioning of the endothelial VLDL receptor and/or VLDL response element discussed in section 5.2.5.

5.5. ASSOCIATION BETWEEN FIBRINOGEN CONCENTRATION AND ALCOHOL CONSUMPTION

The results of the present study show that the fibrinogen concentrations of the PURE study population decreased significantly with moderate alcohol consumption in comparison with concentrations in non-drinkers. However, no further decrease was observed with heavy alcohol consumption. This is in agreement with results from various epidemiological studies on the correlation between fibrinogen concentrations and alcohol consumption in Caucasians, which also show that fibrinogen concentrations decrease with alcohol consumption, and that the lowest fibrinogen concentrations are found in participants who consume moderate amounts of alcohol (Meade *et al.,* 1979; Folsom *et al.*, 1991; Krobot *et al.*, 1992; Lee *et al.,* 1995; Marques-Vidal *et al.*, 1998; Mennen *et al.*, 1999; Yarnell *et al.*, 2000; Mukamal *et al.*, 2001; Wannamethee *et al.*, 2003; Mukamal *et al.*, 2004; Imhof *et al.,* 2004; Shroder *et al.*, 2005; Pomp *et al.*, 2008; Perissinotto *et al.,* 2009; Tolstrup *et al.*, 2009). As mentioned above, there were no

further decreases in fibrinogen concentrations with heavy alcohol consumption. The literature also shows that fibrinogen concentrations plateau or even increase with heavy alcohol consumption (Krobot *et al.*, 1992; Mennen *et al.*, 1999; Mukamal *et al.*, 2001; Imhof *et al.*, 2004; Pomp *et al.*, 2008). Thus, despite higher absolute fibrinogen concentrations in comparison with Caucasians, it seems that the influence of alcohol consumption on fibrinogen concentrations in the black South African PURE population follows the same trend as in Caucasian populations.

The precise mechanisms by which moderate alcohol consumption reduce fibrinogen concentration remain to be elucidated. Fibrinogen, which is produced in the liver, is an acute-phase reactant and is therefore increased with inflammation (Kamath & Lip, 2003; Libby & Ridker, 2006). The mechanism by which moderate alcohol consumption reduces fibrinogen concentration seems to be due to decreased inflammation (Wandler *et al.*, 2008), as moderate alcohol consumption is considered to be anti-inflammatory (Imhof & Koenig, 2003). Wang *et al.* (1999) examined the effect of ethanol on fibrinogen concentrations in rats *in vitro*, and found that alcohol decreased cellular levels of fibrinogen mRNA. These researchers speculated that a possible reason for this decrease may involve inhibited production or activity of interleukin-6 (IL-6), the principal cytokine regulating fibrinogen gene transcription.

5.5.1. Influence of population sub-divisions on the association of alcohol consumption with fibrinogen concentration

Factors which have been shown to influence fibrinogen concentrations were used to sub-divide the participants of the present study into groups. Within these sub-divisions two-way ANCOVAs indicated that neither gender nor urbanisation nor waist circumference nor BMI ranges significantly influenced the association between alcohol consumption and fibrinogen concentration.

Significantly reduced fibrinogen concentrations were observed in participants who consumed moderate amounts of alcohol and in participants who had normal waist circumferences, but not in those who had central obesity. In the BMI-subdivision the

consumption of moderate amounts of alcohol also led to decreased fibrinogen concentrations in participants within the normal and even overweight BMI ranges. However, in abdominally obese and obese (BMI ≥ 30) participants the consumption of alcohol did not decrease fibrinogen concentrations. This may be an indication that the potential for alcohol to protect against CVD by lowering fibrinogen concentration is inhibited in participants who are (abdominally) obese. Since the suggested mechanism through which moderate alcohol consumption decreases fibrinogen concentration is through an anti-inflammatory pathway, a possible explanation for the fact that this reduction in fibrinogen concentration was not seen in (abdominally) obese individuals, might be the larger amount of inflammatory active adipose tissue in (abdominally) obese individuals. It is likely that the pro-inflammatory effect of the adipose tissue counteracts the moderate anti-inflammatory effect of moderate alcohol consumption.

5.6. LIMITATIONS

- Because self-reported data on alcohol consumption are not always reliable, particularly in individuals who consume excessive amounts of alcohol, the use of clinical biomarkers to determine actual amounts consumed would be ideal. The importance of the development of such biomarkers, which can be used to determine alcohol intake across all ranges of alcohol consumption, should be emphasised. The two biomarkers which are currently used, GGT and %CDT, are used mainly to determine alcohol abuse, and not alcohol consumption *per se*. The correlations between these two biomarkers and reported alcohol consumption from quantitative food frequency questionnaires in the PURE population were also very weak and, owing to the lack of availability of biomarkers of alcohol consumption it was decided to use the reported amounts of alcohol only to determine the association between alcohol consumption, PAI-1_{act} and fibrinogen concentration.
- While a QFFQ was used that was validated specifically for this population, the reliability of the reported alcohol intake is not known. It is therefore possible that

the alcohol consumption data obtained from the QFFQ may not be an accurate reflection of the actual alcohol consumption of the participants. It should be noted, however, that despite this uncertainty, the same trends in PAI-1_{act} and fibrinogen concentration in relation to alcohol consumption were observed in this study as have been reported in the literature for Caucasians. This suggests that the reported alcohol intake, although it might not be entirely accurate, is probably a good reflection of the actual intake.

- Unfortunately, the unavailability of data regarding the types of beverages consumed in this population meant that we were unable to investigate associations between specific types of alcoholic beverages and the two haemostatic risk factors, PAI-1_{act} and fibrinogen.
- The design of the QFFQ did not allow us to draw conclusions regarding the frequency of alcohol consumption and therefore we were not able to determine drinking patterns such as binge drinking.
- Owing to the cross-sectional design of the study, we were able only to draw conclusions regarding the relationship between alcohol consumption and PAI-1_{act} and fibrinogen levels, and not to determine causality.
- While we adjusted for several confounders identified in the dataset, there is still the possibility of unknown/unidentified residual confounders that may have been present and which could have affected the results

5.7. CONCLUSION

The main aim of this study was to determine the association between the habitual alcohol consumption of participants of the PURE study with PAI-1_{act} and fibrinogen concentrations of these participants, as well as to determine whether factors known to influence PAI-1 and fibrinogen, may modify this relationship. Specific objectives were:

- To investigate the possibility of GGT and %CDT use as proxy markers in the association between alcohol, fibrinogen and PAI-1_{act}

- To investigate the effect of urbanisation on the association between alcohol consumption, PAI-1_{act} and fibrinogen
- To investigate the association between habitual alcohol consumption and PAI-1_{act} in relation to the 4G/5G polymorphism
- To investigate the association between habitual alcohol consumption and PAI-1_{act} and fibrinogen concentration in relation to body composition (BMI and waist circumference)
- To investigate whether the association between habitual alcohol consumption and PAI-1_{act} is modulated by triglyceride concentration.

In the South African PURE population, PAI-1_{act} was increased in participants with heavy alcohol consumption (when compared to non-drinkers and moderate drinkers) after adjustment for triglyceride concentration and waist circumference. Fibrinogen concentrations decreased significantly with moderate alcohol consumption, after which a plateau was reached. Thus, even though the concentration of fibrinogen is generally higher and PAI-1_{act} lower in black South Africans than in Caucasians, the association between these two haemostatic risk factors and alcohol consumption seems to follow the same pattern as in Caucasian populations. It seems as though obese and abdominally obese individuals who are heavy alcohol consumers, may be at an increased risk for CVD owing to increased PAI-1 which may inhibit fibrinolytic processes.

The possible use of GGT and %CDT has been addressed in Section 5.6, as part of the limitations of the study.

Neither urbanisation nor the 4G/5G polymorphism nor BMI ranges significantly influenced the association between alcohol consumption and PAI-1_{act}. It does seem, however, that normal waist circumference measurements and normal triglyceride concentration may be protective against the increase in PAI-1_{act} associated with heavy alcohol consumption. Our results indicate that possible mechanisms through which

alcohol increases PAI-1_{act} is through altering adipocyte and triglyceride related PAI-1 production. The association between fibrinogen and alcohol consumption was not significantly influenced by gender, urbanisation, waist circumference or BMI range. However, (abdominal) obesity may counteract the moderate anti-inflammatory effect of moderate alcohol consumption, because of the larger amount of inflammatory active adipose tissue in (abdominally) obese participants.

We suggest that ethnic-specific normal healthy ranges for PAI-1 and fibrinogen concentration be developed for use in the black South African population. The higher fibrinogen concentrations observed in black South Africans when compared with ranges for Caucasian populations, may predispose the black South African population to higher risk for CVD, or it may even be that these are the healthy or optimal concentrations for this population. Therefore, the development of an ethnic-specific normal range requires urgent attention.

Further research should focus on elucidating the mechanisms through which alcohol consumption influences PAI-1_{act} and fibrinogen concentration. Another interesting undertaking would be to determine the associations of different types of alcoholic beverages with fibrinogen concentration and PAI-1_{act} in a black South African population. From the literature it seems as though the effect of different alcoholic beverages on these haemostasis markers may vary. Table 2.2 in Chapter 2 indicated that the negative association between fibrinogen and alcohol may be most consistent in wine consumers while the results from studies in which PAI-1 was investigated (Table 2.3) remain largely inconclusive.

6. REFERENCE LIST

AGARWAL, D.P. 2002. Cardioprotective effects of light-moderate consumption of alcohol: a review of putative mechanisms. *Alcohol & alcoholism,* 37(5):409-415.

AJJAN, R. & GRANT, P.J. 2006. Coagulation and atherothrombotic disease. *Atherosclerosis,* 186(2):240-259.

AKINBOBOYE, O., IDRIS, O., AKINBOBOYE, O. & AKINKUGBE, O. 2003. Trends in coronary artery disease and associated risk factors in sub-Saharan Africans. *Journal of human hypertension,* 17(6):381-387.

ALESSI, M.C., PEIRETTI, F., MORANGE, P., HENRY, M., NALBONE, G. & JUHAN-VAGUE, I. 1997. Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. *Diabetes,* 46(5):860-867.

AMIRA, C., NTYINTYANE, L., WILKINSON, D., STEWART, S., BECKER, A., LIBHABER, E. & SLIWA, K. 2006. Emerging epidemic of cardiovascular disease among urban Africans: acute coronary syndrome at Baragwanath Hospital, Soweto. *South African heart journal,* 1(3):7-10.

ANTON, R.F. & MOAK, D.H. 1994. Carbohydrate-deficient transferrin and gamma-glutamyltransferase as markers of heavy alcohol consumption: gender differences. *Journal of alcoholism, clinical & experimental research,* 18(3):747-754.

ANTON, R.F., DOMINICK, C., BIGELOW, M. & WESTBY, C. 2001. Comparison of Bio-Rad %CDT TIA and CDTect as laboratory markers of heavy alcohol use and their relationships with γ-glutamyltransferase. *Clinical chemistry,* 47(10):1769-1775.

ARIËNS, R.A.S., LAI, T., WEISEL, J.W., GREENBERG, C.S. & GRANT, P.J. 2002. Role of factor XIII in fibrin clot formation and effects of genetic polymorphisms. *Blood,* 100(3):743-754.

AVELLONE, G., DI GARBO, V., CAMPISI, D., DE SIMONE, R., RANELI, G., SCAGLIONE, R. & LICATA, G. 2006. Effects of moderate Sicilian red wine consumption on inflammatory biomarkers of atherosclerosis. *European journal of clinical nutrition,* 60(1):41-47.

BARON, P. 2004. A comprehensive guide to preventative blood testing. *Life extension magazine*;6, May.

BAROS, A.M., WRIGHT, T.M., LATHAM, P.K., MILLER, P.M. & ANTON, R.F. 2008. Alcohol consumption, %CDT, GGT and blood pressure change during alcohol treatment. *Alcohol & alcoholism,* 43(2):192-197.

BASTELICA, D., MORANGE, P., BERTHET, B., BORGHI, H., LACROIX, O., GRINO, M., JUHAN-VAGUE, I. & ALESSI, M. 2002. Stromal cells are the main plasminogen activator inhibitor-1 producing cells in human fat: evidence of differences between visceral and subcutaneous deposits. *Arteriosclerosis, thrombosis, and vascular biology*, 22(1):173-178.

BLOMBÄCK, B., BLOMBÄCK, M., CALSSON, K., FATAH, K., HAMSTEN, A. & HESSEL, B. 1992. Fibrin gel structure in health and disease. (*In* Ernst, E., Koenig, W., Lowe, G.D.O. & Meade, T.W., *eds.* Fibrinogen: a "new" cardiovascular risk factor. Oxford : Blackwell. p. 11-18.)

BINDER, B.R., CHRIST, G., GRUBER, F., GRUBIC, N., HUFNAGL, P., KREBS, M., MIHALY, J. & PRAGER, G.W. 2002. Plasminogen activator inhibitor 1: physiological and pathophysiological roles. *News in physiological sciences*, 17(2):56-61.

BOOYSE, F.M., WAIKENS, M.L. & GRENETT, H.E. 1999. Endothelial cell fibrinolysis: transcriptional regulation of fibrinolytic protein gene expression (t-PA, u-PA, and PAI-1) by low alcohol. *Alcoholism: clinical and experimental research*, 23(6):1119-1124.

BORTOLOTTI, F., DE PAOLI, G. & TAGLIARO, F. 2006. Carbohydrate-deficient transferrin (CDT) as a marker of alcohol abuse: a critical review of the literature 2001-2005. *Journal of chromatography B,* 841(1-2):96-109.

BRIEDE, J.J., HEEMSKERK, J.W.M., VAN'T VEER, C., HEMKER, H.C. & LINDHOUT, T. 2001. Contribution of platelet-derived factor Va to thrombin generation on immobilized collagen- and fibrinogen-adherent platelets. *Thrombosis and haemostasis,* 85(3):509-513.

BURZOTTA, F., DI CASTELNUOVO, A., AMORE, C., D'ORAZIO, A., DI BITONDO, R., DONATI, M.B. & IACOVIELLO, L. 1998. 4G/5G promoter PAI-1 gene polymorphism is associated with plasmatic PAI-1 activity in Italians: a model of gene-environment interaction. *Thrombosis and haemostasis,* 79(2):354-358.

CABALLERIA, J. 2003. Current concepts in alcohol metabolism. *Annals of hepatology,* 2(2):60-68.

CABALLERIA, J. 2005. Alcohol metabolism and its metabolic consequences in the liver. *Current medical literature: gastroenterology,* 24(1):1-6.

CESARI, M., SARTORI, M.T., PATRASSI, G.M., VETTORE, S. & ROSSI, G.P. 1999. Determinants of plasma levels of plasminogen activator inhibitor-1 – a study of normotensive twins. *Arteriosclerosis, thrombosis, and vascular biology,* 19(2):316-320.

CHADAREVIAN, R., BRUCKERT, E., DEJAGER, S., PRESBERG, P. & TURPIN, G. 1999. Relationship between triglycerides and factor VIIc and plasminogen activator inhibitor type-1: lack of threshold value. *Thrombosis and haemostasis*, 96(3):175-182.

CHRISTIANSEN, V.J., JACKSON, K.W., LEE, K.N. & MCKEE, P.A. 2007. The effect of a single nucleotide polymorphism on human alpha 2-antiplasmin activity. *Blood*, 109(12):5286-5292.

CLEMENS, D.L. 2006. Use of cultured cells to study alcohol metabolism. *Alcohol research & health,* 29(4):291-295.

CLEOPHAS, T.J. 1999. Wine, beer and spirits and the risk of myocardial infarction: a systematic review. *Biomedicine & pharmacotherapy,* 53(9):417-423.

COLLEN, D. & LIJNEN, H.R. 1991. Basic and clinical aspects of fibrinolysis and thrombolysis. *Blood,* 78(12):3114-3124.

COLLINS, M.A., NEAFSEY, E.J., MUKAMAL, K.J., GRAY, M.O., PARKS, D.A., DAS, D.K. & KORTHUIS, R.J. 2009. Alcohol in moderation, cardioprotection and neuroprotection: epidemiological considerations and mechanistic studies. *Alcoholism: clinical and experimental research,* 33(2):206-219.

COLMAN, R.W., CLOWES, A.W., GEORGE, J.N., HIRSH, J. & MARDER, V.J. 2000. Overview of hemostasis. (*In* Colman, R.W., Hirsh, J., Marder, V.J., Clowes, A.W. & George, J.N., *eds.* Hemostasis and thrombosis: basic principles and clinical practice. 4th ed. Philadelphia: Lippincott Williams & Wilkins. p. 3-20.)

COOK, N.S. & UBBEN, D. 1990. Fibrinogen as a major risk factor in cardiovascular disease. *Trends in pharmacological sciences,* 11(11):444-451.

CRAINICH, P., JENNY, N.S., TANG, Z., ARNOLD, A.M., KULLER, L.H., MANILIO, T., SHARRET, A.R. & TRACY, R.P. 2003. Lack of association of the plasminogen activator inhibitor-1 4G/5G promoter polymorphism with cardiovascular disease in the elderly. *Journal of thrombosis and haemostasis*, 1(8):1799-1804.

DAEPPEN, J.B., SMITH, T.L. & SCHUCKIT, M.A. 1998. Influence of age and body mass index on gamma-glutamyl transferase activity: a 15-year follow-up evaluation in a community sample. *Alcohol, clinical & experimental research*, 22(4):941-944.

DAWSON, S., HAMSTEN, A., WIMAN, B., HENNEY, A. & HUMPHRIES, S. 1991. Genetic variation at the plasminogen activator inhibitor-1 locus is associated with altered levels of plasma plasminogen activator inhibitor-1 activity. *Arteriosclerosis and thrombosis,* 11(1):183-190.

DAWSON, S.J., WIMAN, B., HAMSTEN, A., GREEN, F., HUMPHRIES, S. & HENNEY, A.M. 1993. The two allele sequences of a common polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene respond differently to interleukin-1 in HepG2 cells. *Journal of biological chemistry,* 268(15):10739-10745.

DEEG, M., BAIYEWU, O., GAO, S., OGUNNIYI, A., SHEN, J., GUREJE, O., TAYLOR, S., MURREL, J., UNVERZAGT, F., SMITH-GAMBLE, V., EVANS, R., DICKENS, J., HENDRIE, H. & HALL, K. 2008. A comparison of cardiovascular risk factor biomarkers in African Americans and Yoruba Nigerians. *Ethnicity & disease*, 18(4):427-433.

DE FEO, T.M., FARGION, S., DUCA, L., MATTIOLI, M., CAPPELLINI, M.D., SAMPIETRO, M., CESANA, B.M. & FIORELLI, G. 1999. Carbohydrate deficient transferrin, a sensitive marker of chronic alcohol abuse, is highly influenced by body iron. *Hepatology,* 29(4):658-663.

DELAHOUSSE, B., MAILLOT, F., GABRIEL, I., SCHELLENBERG, F., LAMSSE, F. & GRUEL, Y. 2001. Increased plasma fibrinolysis and tissue-type plasminogen activator/ tissue-type plasminogen activator inhibitor ratios after ethanol withdrawal in chronic alcoholics. *Blood, coagulation and fibrinolysis,* 12(1):59-66.

DELLAS, C. & LOSKUTOFF, D.J. 2005. Historical analysis of PAI-1 from its discovery to its potential role in cell motility and disease. *Thrombosis and haemostasis*, 93(4):631-640.

DEPARTMENT OF HEALTH. 2003. South African demographic and health survey 1998. [Web:] http://www.doh.gov.za/facts/1998/index.html [Date of use: 9 April 2010].

DEPARTMENT OF HEALTH. 2007. South African demographic and health survey 2003. [Web:] http://www.mrc.ac.za/bod/sadhs.htm [Date of use: 9 April 2010].

DIEBOLD, I., KRAICUN, D., BONELLO, S. & GÖRLACH, A. 2008. The 'PAI-1 paradox' in vascular remodelling. *Thrombosis and haemostasis*, 100(6):984-991.

DIMMIT, S.B., RAKIE, V., PUDDEY, I.B., BAKER, R., OOSTRYCK, R., ADAMS, M.J., CHESTERMAN, C.N., BURKE, V. & BEILIN, L.J. 1998. The effects of alcohol on coagulation and fibrinolytic factors: a controlled trial. *Blood, coagulation and fibrinolysis,* 9(1):39-45.

DJOUSSÉ, L., PANKOW, J.S., ARNETT, D.K., ZHANG, Y., HONG, Y., PROVINCE, M.A. & ELLISON, R.C. 2000. Alcohol consumption and plasminogen activator inhibitor type 1: the National Heart, Lung, and Blood Institute family heart study. *American heart journal,* 139(4):704-709.

DONATI, M.B. & IACOVIELLO, L. 2007. Fibrinogen and factor VIIc levels: independent risk factors or markers of coronary disease risk? *Journal of thrombosis and haemostasis,* 5(3):458-460.

ELMÉR, O., GÖRANSSON, G. & ZOUCAS, E. 1984. Impairment of primary hemostasis and platelet function after alcohol ingestion in man. *Haemostasis,* 14(2):223-228.

EL-SAYED, M.S., EASTLAND, P., LIN, X. & RATTU, M.J. 1999. The effect of moderate alcohol ingestion on blood coagulation and fibrinolysis at rest and in response to exercise. *Journal of sport sciences,* 17(6):513-520.

ERIKSSON, P., KALLIN, B., VAN'T HOOFT, F.M., BAVENHOLM, P. & HAMSTEN, A. 1995. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. *Proceedings of the National Academy of Sciences,* 92(6):1851-1855.

ERIKSSON, P., NILSSON, L., KARPE, F. & HAMSTEN, A. 1998. Very-low-density lipoprotein response element in the human plasminogen activator inhibitor-1 gene implicated in the impaired fibrinolysis of hypertriglyceridemia. *Arteriosclerosis, thrombosis, and vascular biology,* 18(1):20-26.

ESTRUCH, R., SACANELLA, E., BADIA, E., ANTÚNEZ, E., NICOLÁS, J.M., FERNÁNDEZ-SOLÁ, J., ROTILIO, D., DE GAETANO, G., RUBIN, E. & URBANO-MÁRQUEZ, A. 2004. Different effects of red wine and gin consumption on inflammatory biomarkers of atherosclerosis: a prospective randomized crossover trial, effects of wine on inflammatory markers. *Atherosclerosis,* 175(1):117-123.

FERGUSON, M.A., GUTIN, B., OWENS, S., LITAKER, M., TRACY, R.P. & ALLISON, J. 1998. Fat distribution and hemostatic measures in obese children. *American journal of clinical nutrition,* 67(6):1136-1140.

FESTA, A., D'AGOSTINO, R., RICH, S.S., JENNY, N.S., TRACY, R.P. & HAFFNER, S.M. 2003. Promoter (4G/5G) plasminogen activator inhibitor-1 genotype and plasminogen activator inhibitor-1 levels in blacks, Hispanics, and non- Hispanic whites: the Insulin Resistance Atherosclerosis Study. *Circulation,* 107(19):2422-2427.

FREDERICK, R., POCHET, L., CHARLIER, C. & MASEREEL, B. 2005. Modulators of the coagulation cascade: focus and recent advances in inhibitors of tissue factor, factor VIIa and their complex. *Current medicinal chemistry,* 12(4):397-417.

FOLSOM, A.R., WU, K.K., DAVIS, C.E., CONLAN, M.G., SORLIE, P.D. & SZKLO, M. 1991. Population correlates of plasma fibrinogen and factor VII, putative cardiovascular risk factors. *Atherosclerosis,* 91(3):191-205.

FOLSOM, A.R. 2001. Hemostatic risk factors for atherothrombotic disease: an epidemiologic view. *Thrombosis and haemostasis,* 86(1):366-373.

GABAY, C. & KUSHNER, I. 1999. Acute-phase proteins and other systemic responses to inflammation. *New England journal of medicine*, 340(6):448-454.

GORINSTEIN, S., ZEMSER, M., LICHMAN, I., BEREBI, A., KLEIPFISH, A., LIBMAN, I., TRAKHTENBERG, S. & CASPI, A. 1997. Moderate beer consumption and the blood coagulation in patients with coronary artery disease. *Journal of internal medicine,* 241(1):47-51.

GRENETT, H.E., AIKENS, M.L., TABENGWA, E.M., DAVIS, G.C. & BOOYSE, F.M. 2000. Ethanol downregulates transcription of the PAI-1 gene in cultured human endothelial cells. *Thrombosis research,* 97(4):247-255.

GREYLING, A., PIETERS, M., HOEKSTRA, T., OOSTHUIZEN, W. & SCHUTTE, A.E. 2007. Differences in the association of PAI-1 activity with the metabolic syndrome between African and Caucasian women. *Nutrition, metabolism & cardiovascular diseases*, 17(7):499-507.

HAMSTEN, A., WIMAN, B., DEFAIRE, U. & BLOMBÄCK, M. 1985. Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. *New England journal of medicine,* 313(25):1557-1563.

HAMSTEN, A., DE FAIRE, U., WALLDIUS, G., DAHLEN, G., SZAMOSI, A., LANDOU, C., BLOMBÄCK, M. & WIMAN, B. 1987. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. *Lancet,* 330(8549):3-9.

HANSEN, A.S., MARCKMANN, P., DRAGSTED, L.O., FINNÉ NIELSEN, I.L., NIELSEN, S.E. & GRØNBAEK, M. 2005. Effect of red wine and red grape extract on blood lipids, haemostatic factors, and other risk factors for cardiovascular disease. *European journal of clinical nutrition,* 59(3):449-455.

HARPEL, P.C., CHANG, T. & VERDERBER, E. 1985. Tissue plasmingen activator and urokinase mediate the binding of glu-plasminogen to plasma fibrin I – evidence for new binding sites in plasmin-degraded fibrin I. *The journal of biological chemistry,* 260(7):4432-4440.

HAWKINS, M.A. 2004. Markers of increased cardiovascular risk: are we measuring the most appropriate parameters? *Obesity research*, 12(Suppl. 2):107-114.

HENDRIKS, H.F.J., VEENSTRA, J., VELTHUIS-TE WIERIK, E.J.M., SCHAAFSMA, G. & KLUFT, C. 1994. Effect of moderate dose of alcohol with evening meal on fibrinolytic factors. *British medical journal*, 308(6935):1003-1006.

HENRY, M., CHOMIKI, N., SCARABIN, P.Y., ALESSI, M.C., PEIRETTI, F., ARVEILER, D., FERRIERES, J., EVANS, A., AMOUYEL, P., POIRIER, O., CAMBIEN, F. & JUHAN-VAGUE, I. 1997. Five frequent polymorphisms of the PAI-1 gene – lack of association between genotypes, PAI activity, and triglyceride levels in a healthy population. *Arteriosclerosis, thrombosis, and vascular biology*, 17(5):851-858.

HIGGINS, D.L. & VEHAR, G.A. 1987. Interaction of one-chain and two-chain tissue plasminogen activator with intact and plasmin-degraded fibrin. *Biochemistry,* 26(24):7786-7791.

HOEKSTRA, T., GELEIJNSE, J.M., SCHOUTEN, E.G. & KLUFT, C. 2004. Plasminogen activator inhibitor-type 1: its plasma determinants and relation with cardiovascular risk. *Thrombosis and haemostasis,* 91(5):861-872.

HOFFMAN, M. & MONROE, D.M. 2001. A cell-based model of hemostasis. *Thrombosis and haemostasis,* 85(6):958-965.

HOYLAERTS, M., RIJKEN, R.C., LIJNEN, H.R. & COLLEN, D. 1982. Kinetics of the activation of plasminogen by human tissue plasminogen activator – role of fibrin. *The journal of biological chemistry,* 257(6):2912-2919.

IMHOF, A. & KOENIG, W. 2003. Alcohol inflammation and coronary heart disease. *Addiction biology,* 8(3):271-277.

IMHOF, A., WOODWARD, M., DOERING, A., HELBECQUE, N., LOEWEL, H., AMOUYEL, P., LOWE, G.D.O. & KOENIG, W. 2004. Overall alcohol intake, beer, wine, and systemic markers of inflammation in Western Europe: results from three MONICA samples (Augsburg, Glasgow, Lille). *European heart journal,* 25(23):2092-2100.

INTERNATIONAL CENTER FOR ALCOHOL POLICIES. 2003. International drinking guidelines. [Web:] http://www.icap.org/Publications/ICAPReports/tabid/75/Default.aspx [Date of use: 18 Jan. 2010].

JACKSON, E., SKERRETT, P.J. & RIDKER, P.M. 2000. Epidemiology of arterial thrombosis. (*In* Colman, R.W., Hirsh, J., Marder, V.J., Clowes, A.W. & George, J.N., *eds.* Hemostasis and thrombosis: basic principles and clinical practice. 4th ed. Philadelphia: Lippincott Williams & Wilkins. p. 1179-1196.)

JAMES, W.P.T. & RALPH, A. 2000. Alcohol: its metabolism and effects. (*In* Garrow, J.S., James, W.P.T., Ralph, A., *eds.* Human nutrition and dietetics. 10th ed. Edinburgh: Churchill Livingstone. p. 121-135.)

JAMES, S., VORSTER, H.H., VENTER, C.S., KRUGER, H.S., NELL, T.A., VELDMAN, F.J. & UBBINK, J.B. 2000. Nutritional status influences plasma fibrinogen concentration: evidence from the THUSA survey. *Thrombosis research*, 98(5):383-394.

JUHAN-VAGUE, I., MORANGE, P.E. & ALESSI, M. 2002. The insulin resistance syndrome: implications for thrombosis and cardiovascular disease. *Pathophysiology of haemostasis and thrombosis*, 32(5-6):269-273.

JUHAN-VAGUE, I., ALESSI, M., MAVRI, A. & MORANGE, P.E. 2003. Plasminogen activator inhibitor-1, inflammation, obesity, insulin resistance and vascular risk. *Journal of thrombosis and haemostasis*, 1(7):1575-1579.

JERLING, J.C., VORSTER, H.H., OOSTHUIZEN, W., SILVIS, N. & VENTER, C.S. 1994. Differences in plasminogen activator inhibitor 1 activity between blacks and whites may be diet related. *Haemostasis*, 24(6):364-368.

KAMATH, S. & LIP, G.Y.H. 2003. Fibrinogen: biochemistry, epidemiology and determinants. *Q J med*, 96(10):711-729.

KEILLOR, J.W., CASTONGUAY, R. & LHERBET, C. 2005. Gamma-glutamyl transpeptidase substrate specificity and catalytic mechanism. *Methods in enzymology,* 401:449-467.

KIECHL, S., WILLEIT, J., RUNGGER, G., EGGER, G., OBERHOLLENZER, F. & BONORA, E. 1998. Alcohol consumption and atherosclerosis: what is the relation? *Stroke*, 29(5):900-907.

KIVINIEMI, T.O., SARASTE, A., LEHTIMÄKI, T., TOIKKA, J.O., SARASTE, M., RAIAKARI, O.T., PÄRKKÄ, HARTIALA, J.J., VIIKARI, J. & KOSKENVUO, J.W. 2009. High dose of red wine elicits enhanced inhibition of fibrinolysis. *European journal of cardiovascular prevention and rehabilitation*, 16(2):161-163.

KOENIG, W. 2003. Fibrin(ogen) in cardiovascular disease: an update. *Thrombosis and haemostasis*, 89(4):601-609.

KOHLER, H.P. & GRANT, P.J. 2000. Plasminogen-activator inhibitor type-1 and coronary artery disease. *The New England journal of medicine*, 342(24):1792-1801.

KREBS, M., GEIGER, M., POLAK, K., VALES, A., SCHMETTERER, L., WAGNER, O.F., WALDÄUSL, W., BINDER, B.R. & RODEN, M. 2003. Increased plasma levels of plasminogen activator inhibitor-1 and soluble vascular cell adhesion molecule after triacylglycerol infusion in man. *Thrombosis and haemostasis*, 90(3):422-428.

KRISHNAMURTI, C., TANG, D.B., BARR, C.F. & ALVING, B.M. 1988. Plasminogen activator and plasminogen activator inhibitor activities in a reference population. *American journal of clinical pathology*, 89(6):747-752.

KROBOT, K., HENSE, H.W., CREMER, P., EBERLE, E. & KEIL, U. 1992. Determinants of plasma fibrinogen: relation to body weight, waist-to-hip ratio, smoking, alcohol, age, and sex. Results from the second MONICA Augsburg survey 1989-1990. *Arteriosclerosis, thrombosis, and vascular biology,* 12(7):780-788.

LACROIX, K.A., BEAN, C., BOX, L. & WAGNER, K. 1996. A study of the fibrinolytic response in healthy men and women following a brief exposure to venous occlusion. *Thrombosis research*, 81(1):133-143.

LEE, A.J., FLANAGAN, P.A., RUMLEY, A., FOWKES, F.G.R. & LOWE, G.D.O. 1995. Relationship between alcohol intake and tissue plasminogen activator antigen and other haemostatic factors in the general population. *Fibrinolysis,* 8:49-54.

LIBBY, P. & RIDKER, P.M. 2006. Inflammation and atherothrombosis, from population biology and bench research to clinical practice. *Journal of the American College of Cardiology*, 48(9):33-46.

LIEBER, C.S. 1997. Ethanol metabolism, cirrhosis and alcoholism. *Clinica chimica acta,* 257(1):59-48.

LIEBER, C.S. 2000. Alcohol and the liver: metabolism of alcohol and its role in hepatic and extrahepatic diseases. *The Mount Sinai journal of medicine,* 67(1):84-94.

LOHMAN, T.G., ROCHE, A.F. & MARTORELL, R. 1988. Anthropometric standardization reference manual. Champaign, Illinois: Human kinetic books. 177 p.

LOSKUTOFF, D.J., SAWDEY, M., KEETON, M. & SCHNEIDERMAN, J. 1993. Regulation of PAI-1 gene expression *in vivo*. *Thrombosis and haemostasis*, 70(1):135-137.

LUCAS, D.L., BROWN, R.A., WASSEF, M. & GILES, T.D. 2005. Alcohol and the cardiovascular system: research challenges and opportunities. *Journal of the American College of Cardiology,* 45(12):1916-1924.

MA, Y., RYU, J.S., DULAY, A., SEGAL, M. & GULLER, S. 2002. Regulation of plasminogen activator inhibitor (PAI) - 1 expression in a human trophoblast cell line by glucocorticoid (GC) and transforming growth factor (TGF) – β. *Placenta*, 23(10):727-734.

MARGAGLIONE, M., GRANDONE, E., VECCHIONE, G., CAPPUCCI, G., GIULIANI, N., COLAIZZO, D., CELENTANO, E., PANICO, S. & DI MINNO, G. 1997. Plasminogen activator inhibitor (PAI-1) antigen plasma levels in subjects attending a metabolic ward: relation of polymorphisms PAI-1 and angiotensin converting enzyme (ACE) genes. *Arteriosclerosis, thrombosis, and vascular biology*, 17(10):2082-2087.

MARGAGLIONE, M., CAPPUCCI, G., D'ADDEDDA, M., COLAIZZO, D., GIULIANI, N., VECCHIONE, G., MASCOLO, G., GRANDONE, E. & DI MINNO, G. 1998. PAI-1 plasma levels in a general population without clinical evidence of atherosclerosis: relation to environmental and genetic determinants. *Arteriosclerosis, thrombosis, and vascular biology,* 18(4):562-567.

MARMOT, M. & BRUNNER, E. 1991. Alcohol and cardiovascular disease: the status of the U-shaped curve. *British medical journal,* 303(6802):565-568.

MARQUES-VIDAL, P., CAMBOU, J., NICAUD, V., LUC, G., EVANS, A., ARVEILER, D., BINGHAM, A. & CAMBIEN, F. 1995. Cardiovascular risk factors and alcohol consumption in France and Northern Ireland. *Atherosclerosis,* 115(2):225-232.

MARQUES-VIDAL, P., MONTAYE, M., HAAS, B., BINGHAM, A., EVANS, A., JUHAN-VAGUE, I., FERRIÈRES, J., LUC, G., AMOUYEL, P., ARVEILER, D., YARNELL, J., RUIDAVETS, J.B., SCARABIN, P. & DUCIMETIÈRE, P. 2001. Relationships between alcoholic beverages and cardiovascular risk factor levels in middle-aged men, the PRIME study. *Atherosclerosis,* 157(2):431-440.

MAY, P.A., GOSSAGE, J.P., MARIS, A., ADNAMS, C.M., HOYME, H.E., JONES, K.L., ROBINSON, L.K., KHAOLE, N.C.O., SNELL, C., KALBERG, W.O., HENDRICKS, L., BROOKE, L., STELLAVATO, C. & VILJOEN, D.L. 2007. The epidemiology of fetal alcohol syndrome and partial FAS in a South African community. *Drug and alcohol dependence*, 88(2-3):259-271.

MCORMACK, L.J., SEMPLE, J.I., STICKLAND, M.H., BARRETT, J.H. & GRANT, P.J. 1998. The effect of number of days in culture and plasminogen activator inhibitor-1 (PAI-1) 4G/5G genotype on PAI-1 antigen release by cultured human umbilical vein endothelial cells. *Thrombosis research*, 92(5):199-206.

MCCONNELL, M.V., VAVOURANAKS, I., WU, L.L., VAUGHAN, D.E. & RIDKER, P.M. 1997. Effect of a single, daily alcoholic beverage on lipid and hemostatic markers of cardiovascular risk. *The American journal of cardiology,* 80(9):1226-1228.

MEADE, T.W., CHAKRABARTI, R., HAINES, A.P., NORTH, W.R.S. & STIRLING, Y. 1979. Characteristics affecting fibrinolytic activity and plasma fibrinogen concentrations. *British medical journal*, 1(6157):153-156.

MEADE, T.W., RUDDOCK, V., STIRLING, Y., CHAKRABARTI, R. & MILLER, G.J. 1993. Fibrinolytic activity, clotting factors, and long term incidence of ischaemic heart disease in the Northwick Park heart study. *Lancet*, 342(8879):1076-1079.

MEDVED, L., TSURUPA, G. & YAKOVLEV, S. 2001. Conformational changes upon conversion of fibrinogen into fibrin – the mechanisms of exposure of cryptic sites. (*In* Nieuwenhuizen, W., Mosesson, M.W. & de Maat, M.P.M., *eds*. Annals of the New York Academy of Sciences, 936. New York, NY: The New York Academy of Sciences. p. 185-204.)

MELONI, J.N. & LARANJEIRA, R. 2004. The social and health burden of alcohol abuse. *Revista Brasileira psiquiatria,* 26(1):7-10.

MENNEN, L.I., BALKAU, B., VOL, S., CACÈS, E. & ESCHWÈGE, E. 1999a. Fibrinogen: a possible link between alcohol consumption and cardiovascular disease? *Arteriosclerosis, thrombosis, and vascular biology,* 19(4):887-892.

MENNEN, L.I., BALKAU, B., CHARLES, M., D'HOUR, A., LE MAUFF, J. & THE D.E.S.I.R. STUDY GROUP. 1999b. Gender differences in the relation between fibrinogen, tissue-type plasminogen activator antigen and markers of insulin resistance: effects of smoking. *Thrombosis and haemostasis*, 82(3):1106-1111.

MENGES, T., HERMANS, P.W., LITTLE, S.G., LANGEVELD, T., BÃNING O., ENGEL, J., SLUIJTER, M., DE GROOT, R. & HEMPELMAN, G. 2001. Plasminogen-activator-inhibitor-1 4G/5G promoter polymorphism and prognosis of severely injured patients. *Lancet*, 357(9262):1096-1097.

MERTENS, I., & VAN GAAL, L.F. 2002. Obesity, haemostasis and the fibrinolytic system. *Obesity reviews,* 3(2):85-101.

MEZZANO, D. 2004. Distinctive effects of red wine and diet on haemostatic cardiovascular risk factors. *Biological research,* 37(2):217-224.

MINAMI, J., TODOROI, M., YOSHII, M., MITA, S., NISHIKIMI, T., ISHIMITSU, T. & MATSUOKA, H. 2002. Effect of smoking cessation or alcohol restriction on metabolic and fibrinolytic variables in Japanese men. *Clinical science,* 103(2):117-122.

MONROE, D.M. & HOFFMAN, M. 2006. What does it take to make the perfect clot? *Arteriosclerosis, thrombosis and vascular biology,* 26(1):41-48.

MOSNIER, L.O. & BOUMA, B.N. 2006. Regulation of fibrinolysis by thrombin activatable fibrinolysis inhibitor, an unstable carboxypetidase B that unites the pathways of coagulation and fibrinolysis. *Arteriosclerosis, thrombosis, and vascular biology,* 26:2445-2453.

MUKAMAL, K.J., JADHAV, P.P., D'AGOSTINO, R.B., MASSARO, J.M., MITTLEMAN, M.A., LIPINSKA, I., SUTHERLAND, P.A., MATHENEY, T., LEVY, D., WILSON, P.W.F., ELLISON, R.C., SILBERSHATZ, H., MULLER, J.E. & TOFLER, G.H. 2001. Alcohol consumption and hemostatic factors: analysis of the Framingham offspring cohort. *Circulation,* 104(12):1367-1373.

MUKAMAL, K.J., CUSHMAN, M., MITTLEMAN, M.A., TRACY, R.P. & SISCOVICK, D.S. 2004. Alcohol consumption and inflammatory markers in older adults: the Cardiovascular Health Study. *Atherosclerosis,* 173(1):79-87.

MUNDT, M.P., KRAUS, C.K. & FLEMING, M.F. 2004. Potential drug interactions with the carbohydrate-deficient transferrin (%CDT) test, a new alcohol biomarker. *Pharmacotherapy,* 24(7):831-837.

MURRAY, C.J.L. & LOPEZ, A.D. 1996. Alternative visions of the future: projecting mortality and disability, 1999-2020. (*In*: Murray, C.J.L., Lopez, A.D., *eds.* The global burden of disease. S.l.: Harvard School of Public Health on behalf of the WHO and the World Bank. p. 325-395.)

NAGY, L.E. 2004. Molecular aspects of alcohol metabolism: transcription factors involved in early ethanol-induced liver injury. *Annual review of nutrition,* 24(1):55-78.

NARAN, N.H., CHETTY, N. & CROWTHER, N.J. 2008. The influence of metabolic syndrome components on plasma PAI-1 concentrations is modified by the PAI-1 4G/5G genotype and ethnicity. *Atherosclerosis*, 196(1):155-163.

NGUYEN, X.T., LANE, J. & SMITH, B.R. 2009. Changes in inflammatory biomarkers across weight classes in a representative US population: a link between obesity and inflammation. *Journal of gastrointestinal surgery*, 13(7):1205-1212.

NIEMELÄ, O. 2007. Biomarkers in alcoholism. *Clinica chimica acta,* 377(1-2):39-49.

NIENABER, C., PIETERS, M., KRUGER, S.H., STONEHOUSE, W. & VORSTER, H.H. 2008. Overfatness, stunting and physical inactivity are determinants of plasminogen activator inhibitor-1 activity, fibrinogen, and thrombin-antithrombin complex in African adolescents. *Blood coagulation and fibrinolysis,* 19(5):361-368.

NILSSON, L., BANFI, C., DICZFALUSY, U., TREMOLI, E., HAMSTEN, A. & ERIKSSON, P. 1998. Unsaturated fatty acids increase plasminogen activator inhibitor-1 expression in endothelial cells. *Arteriosclerosis, thrombosis, and vascular biology*, 18(11):1679-1685.

NILSSON, L., GAFVELS, M., MUSAKKA, L., ENSLER, K., STRICKLAND, D.K., ANGELIN, B., HAMSTEN, A. & ERIKSSON, P. 1999. VLDL activation of plasminogen activator inhibitor-1 (PAI-1) expression: involvement of the VLDL receptor. *Journal of lipid research*, 40(5):913-919.

NORBERG, A., JONES, A.W., HAHN, R.G. & GABRIELSSON, J.L. 2003. Role of variability in explaining ethanol pharmacokinetics: research and forensic applications. *Clinical pharmacokinetics,* 42(1):1-31.

NUMMINEN, H., SYRJÄLÄ, M., BENTHIN, G., KASTE, M. & HILLBOM, M. 2000. The effect of acute ingestion of a large dose of alcohol on the hemostatic system and its circadian variation. *Stroke,* 31(6):1269-1273.

PANAHLOO, A., MOHAMED-ALI, V., LANE, A., GREEN, F., HUMPHRIES, S.E. & YUDKIN, J.S. 1995. Determinants of plasminogen activator inhibitor 1 activity in treated NIDDM and its relation to a polymorphism in the plasminogen activator inhibitor 1 gene. *Diabetes,* 44(1):37-42.

PARK, H.S., PARK, J.Y. & YU, R. 2005. Relationship of obesity and visceral adiposity with concentrations of CRP, TNF-α and IL-6. *Diabetes research and clinical practice*, 69(1):29-35.

PARRY, C.D.H., PLUDDEMANN, A., STEYN, K., BRADSHAW, D., NORMAN, R. & LAUBSCHER, R. 2005. Alcohol use in South Africa: findings from the first demographic and health survey. *Journal of studies on alcohol,* 66(1):91-97.

PATON, A. 2005. Alcohol in the body. *British medical journal,* 330:85-87.

PAWAN, G.L.S. 1972. Metabolism of alcohol (ethanol) in man. *Proceedings of the Nutrition Society,* 31(2):83-89.

PEGORARO, R.J., HIRA, B., ROM, L. & MOODLEY, J. 2003. Plasminogen activator inhibitor type 1 (PAI-1) and platelet glycoprotein IIIa (PGIIIa) polymorphisms in black South Africans with pre-eclampsia. *Acta obstetricia et gynecologica scandinavica,* 82(4):313-317

PELLEGRINI, N., PARETI, F.I., STABILE, F., BRUSAMOLINO, A. & SIMONETTI, P. 1996. Effects of moderate consumption of red wine on platelet aggregation and haemostatic variables in healthy volunteers. *European journal of clinical nutrition,* 50(4):209-213.

PERISSINOTTO, E., BUJA, A., MAGGI, S., ENZI, G., MANZATO, E., SCAFATO, E., MASTRANGELO, G., FRIGO, A.C., COIN, A., CREPALDI, G. & SERGI, G. 2009. Alcohol consumption and cardiovascular risk factors in older lifelong wine drinkers: The Italian longitudinal study on ageing. *Nutrition, metabolism & cardiovascular disease* (In press).

PEVERILL, R.E., TEEDE, H.J., MALAN, E., KOTSOPOULOS, D., SMOLICH, J.J. & MCGRATH, B.P. 2007. Relationship of waist and hip circumference with coagulation and fibrinolysis in postmenopausal women. *Clinical science,* 113(9):383-391.

PIETERS, M., COVIC, N., LOOTS, D.T., VAN DER WESTHUIZEN, F.H., VAN ZYL, D.G., RHEEDER, P., JERLING, J.C. & WEISEL, J.W. 2006. The effect of glycaemic control on fibrin network structure of type 2 diabetic subjects. *Thrombosis and haemostasis,* 96(5):623-629.

PIETERS, M. & VORSTER, H.H. 2008. Nutrition and hemostasis: a focus on urbanization in South Africa. *Molecular nutrition and food research,* 52(1):1-9.

PISA, P., VORSTER, H.H., KRUGER, A., MARGETTS, B.M. & LOOTS, D.T. Percentage carbohydrate deficient transferrin (%CDT) nor gamma glutamyl transferase (GGT) are good markers for alcohol consumption in an African population in transition. *Annals of nutrition and metabolism* (In press).

PIZZO, S.V. & WU, S.M. 2000. α-Macroglobulins and kunins. (*In* Colman, R.W., Hirsh, J., Marder, V.J., Clowes, A.W. & George, J.N., *eds.* Hemostasis and thrombosis: basic principles and clinical practice. 4th ed. Philadelphia: Lippincott Williams & Wilkins. p. 367-379.)

POMP, E.R., ROSENDAAL, F.R. & DOGGEN, C.J.M. 2008. Alcohol consumption is associated with a decreased risk of venous thrombosis. *Thrombosis and haemostasis,* 99(1):59-63.

PUUKA, K., HIETALA, J., KOIVISTO, H., ANTTILA, P., BLOIGU, R. & NIEMELA, O. 2006. Age-related changes of serum GGT activity and the assessment of ethanol intake. *Alcohol & alcoholism,* 41(5):522-527.

RANA, J.S., ARSENAULT, B.J., DESPRÉS, J., CÔTÉ, M., TALMUD, P.J., NINIO, E., JUKEMA, J.W., WAREHAM, N.J., KASTELEIN, J.J.P., KHAW, K. & BOEKHOLDT, S.M. 2009. Inflammatory biomarkers, physical activity, waist circumference, and risk of future coronary heart disease in healthy men and women. *European heart journal* (In press).

RIJKEN, D.C. & LIJNEN, H.R. 2008. New insights into the molecular mechanisms of the fibrinolytic system. *Journal of thrombosis and haemostasis,* 7(1):4-13.

ROSENSON, R.S. & KOENIG, W. 2003. Utility of inflammatory markers in the management of coronary artery disease. *American journal of cardiology*, 92(1A):10-18.

SADLER, P.W. 2007. Alcohol and alcoholism. Department of Forensic Medicine, University of Dundee.

SAKATA, Y. & AOKI, N. 1982. Significance of cross-linking of α2-plasmin inhibitor to fibrin in inhibition of fibrinolysis and in hemostasis. *Journal of clinical investigation,* 69(3):536-542.

SALEM, R.O. & LAPOSATA, M. 2005. Effects of alcohol on hemostasis. *American journal of clinical pathology*, 123(Suppl. 1):1-10.

SARTORI, M.T., VETTORE, R., DE PERGOLA, G., DE MITRIO, V., SAGGIORATO, G., DELLA MEA, P., PATRASSI, G.M., LOMBARDI, A.M., FABRIS, R. & GIROLAMI, A. 2001. Role of the 4G/5G polymorphism of PAI-1 gene promoter on PAI-1 levels in obese patients: influence of fat distribution and insulin-resistance. *Thrombosis and haemostasis*, 86(5):1161-1169.

SASAKI, A., KURISO, A., OHNO, M. & IKEDA, Y. 2001. Overweight/ obesity, smoking, and heavy alcohol consumption are important determinants of plasma PAI-1 levels in healthy men. *The American journal of the medical sciences,* 322(1):19-23.

SCHRÖDER, H., FERRÁNDEZ, O., CONDE, J.J., SÁNCHEZ-FONT, A. & MARRUGAT, J. 2005. Cardiovascular risk profile and type of alcohol beverage consumption: a population-based study. *Annals of nutrition and metabolism,* 49(2):100-106.

SEITZ, H.K., GARTNER, U., EGERER, G. & SIMANOWSKI, U.A. 1994. Ethanol metabolism in the gastrointestinal tract and its possible consequences. *Alcohol and alcoholism supplement*, 2:157-162.

SIERKSMA, A., VAN DER GAAG, M.S., SCHAAFSMA, G., KLUFT, C., BAKKER, M. & HENDRIKS, H.F.J. 2001. Moderate alcohol consumption and fibrinolytic factors of pre- and postmenopausal women. *Nutrition research,* 21(1-2):171-181.

SIERKSMA, A., VAN DER GAAG, M.S., KLUFT, C. & HENDRIKS, H.F.J. 2002. Moderate alcohol consumption reduces plasma C-reactive protein and fibrinogen levels; a randomized, diet-controlled intervention study. *European journal of clinical nutrition,* 56(11):1130-1136.

SKURK, T. & HAUNER, H. 2004. Obesity and impaired fibrinolysis: role of adipose production of plasminogen activator inhibitor-1. *International journal of obesity*, 28(11):1357-1364.

SLIWA, K., WILKINSON, D., HANSEN, C., NTYINTYANE, L., TIBAZARWA, K., BECKER, A. & STEWART, S. 2008. Spectrum of heart disease and risk factors in a black urban population in South Africa (the Heart of Soweto study): a cohort study. *Lancet*, 371(9616):915-922.

STANDEVEN, K.F., ARIËNS, R.A.S. & GRANT, P.J. 2005. The molecular physiology and pathology of fibrin structure/ function. *Blood reviews,* 19(5):275-288.

STAUBER, R.E., JAUK, B., FICKERT, P. & HAUSLER, M. 1996. Increased carbohydrate-deficient transferrin during pregnancy: relation to sex hormones. *Alcohol & alcoholism,* 31(4):389-392.

STEGNAR, M. & PENTEK, M. 1993. Fibrinolytic response to venous occlusion in healthy subjects: Relationship to age, gender, body weight, blood lipids and insulin. *Thrombosis and haemostasis,* 69(1):81-92.

STIKO-RAHM, A., WIMAN, B., HAMSTEN, A. & NILSSON, J. 1990. Secretion of plasminogen activator inhibitor-1 from cultured human umbilical vein endothelial cells is induced by very low density lipoprotein. *Arteriosclerosis*, 10(6):1067-1073.

STRACHAN, K. 1999. Policy in progress -- a profile of alcohol in South Africa. *Health Systems Trust update,* 43:5-14.

SUENSON, E., LÜTZEN, O. & THORSEN, S. 1984. Initial plasmin-degradation of fibrin as the basis of a positive feed-back mechanism in fibrinolysis. *European journal of biochemistry,* 140(3):513-522.

SZABO, G., VERMA, B.K., FOGARASI, M. & CATALANO, D.E. 1992. Induction of transforming growth factor-beta and prostaglandin E_2 production by ethanol in human monocytes. *Journal of leukocyte biology*, 52(6):602-610.

TEO, K., CHOW, C.K., VAZ, V., RANGARAJAN, S. & YUSUF, S. 2009. The Prospective Urban Rural Epidemiology (PURE) study: examining the impact of societal influences on chronic noncommunicable diseases in low-, middle-, and high-income countries. *American heart journal*, 158(1):1-7.

THELWELL, C. & LONGSTAFF, C. 2007. The regulation by fibrinogen and fibrin of tissue plasminogen activator kinetics and inhibition by plasminogen activator inhibitor 1. *Journal of thrombosis and haemostasis,* 5(4):804-811.

TJARNLÜND, A., ANDERSSON, M., JOOD, K., LANDENVALL, P. & JERN, C. 2003. Rapid genotyping of haemostatic gene polymorphisms using the 5' nuclease assay. *Thrombosis and haemostasis,* 89(5):936-942.

TOLSTRUP, J.S., GRØNBÆK, M. & NORDESTGAARD, B.G. 2009. Alcohol intake, myocardial infarction, biochemical risk factors, and alcohol dehydrogenase genotypes. *Circulation: cardiovascular genetics,* 2(5):507-514.

TRAN-THANG, C., KRUITHOF, E.K.O & BACHMANN, F. 1984. Tissue-type plasminogen activator increases the binding of glu-plasminogen to clots. *Journal of clinical investigation*, 74(6):2009-2016.

TOUSOULIS, D., NTARLADIMAS, I., ANTONIADES, C., VASILIADOU, C., TENTOLOURIS, C., PAPAGEORGIOU, N., LATSIOS, G. & STEFANADIS, C. 2008. Acute effects of different alcoholic beverages on vascular endothelium, inflammatory markers and thrombosis fibrinolytic system. *Clinical nutrition,* 27(4):594-600.

UNAIDS/WHO. 2004. Policy statement on HIV testing. [Web:] http://www.who.int/hiv/pub/vct/en/hivtestingpolicy04.pdf [Date accessed: 23 March 2010].

VAN DE WIEL, A., VAN GOLDE, P.M., KRAAIJENHAGEN, R.J., VON DEM BORNE, P.A.K., BOUMA, B.N. & ART, H.C. 2001. Acute inhibitory effect of alcohol on fibrinolysis. *European journal of clinical investigation,* 31(2):164-170.

VAN HARMELEN, V., WAHRENBERG, H., ERIKSSON, P. & ARNER, P. 2000. Role of gender and genetic variance in plasminogen activator inhibitor-1 secretion from human adipose tissue. *Thrombosis and haemostasis*, 83(2):304-308.

VAN HEERDEN, I.V. & PARRY, C.D.H. 2001. If you drink alcohol, drink sensibly. *South African journal of clinical nutrition,* 14(3):S71-77.

VASSE, M., COLLET, J.P., SORIA, J., MIRSHAHI, S.S., VANNIER, J. & SORIA, C. 1994. Fibrinogen, a vascular risk factor: a simple marker or a real cause of vascular lesion? *Thrombosis research*, 75(3):349-352.

VEENSTRA, J., KLUFT, C., OCKHUIZEN, T.H., VD POL, H., WEDEL, M. & SCHAAFSMA, G. 1990. Effects of moderate alcohol consumption on platelet function, tissue-type plasminogen activator and plasminogen activator inhibitor. *Thrombosis and haemostasis*, 63(3):345-348.

VIDAL, F., PEREZ, J., MORANCHO, J., PINTO, B. & RICHART, C. 1990. Hepatic alcohol dehydrogenase activity in alcoholic subjects with and without liver disease. *Gut,* 31(6):707-711.

VOLPATO, S., PAHOR, M., FERRUCCI, L., SIMONSICK, E.M., GURALNIK, J.M., KRITCHEVSKY, S.B., FELLIN, R. & HARRIS, T.B. 2004. Relationship of alcohol intake with inflammatory markers and plasminogen activator inhibitor-1 in well-functioning older adults: the Health, Ageing, and Body Composition study. *Circulation,* 109(5):607-612.

VORSTER, H.H., SILVIS, N., VENTER, C.S., VAN RYSSEN, J.J., HUISMAN, H., VAN EEDEN, T.S. & WALKER, A.R.P. 1987. Serum cholesterol, lipoproteins, and plasma coagulation factors in South African blacks on a high-egg but low-fat intake. *American journal of clinical nutrition*, 46(1):52-57.

VORSTER, H.H., JERLING, J.C., STEYN, K., BADENHORST, C.J., SLAZUS, W., VENTER, C.S., JOOSTE, P.L. & BOURNE, L.T. 1998. Plasma fibrinogen of black South Africans: the BRISK study. *Public health nutrition*, 1(3):169-176.

VORSTER, H.H. 2002. The emergence of cardiovascular disease during urbanisation of Africans. *Public health nutrition*, 5(1A):239-243.

WANDLER, A., BRUUN, J.M., NIELSEN, M.P. & RICHELSEN, B. 2008. Ethanol exerts anti-inflammatory effects in human adipose tissue *in vitro*. *Molecular and cellular endocrinology*, 296(1-2):26-31.

WANG, Z., BARKER, T.H. & FULLER, G.M. 1999. Alcohol at moderate levels decreases fibrinogen expression *in vivo* and *in vitro*. *Alcoholism: clinical and experimental research*, 23(12):1927-1932.

WANNAMETHEE, S.G., LOWE, G.D.O., SHAPER, G., WHINCUP, P.H., RUMLEY, A., WALKER, M. & LENNON, L. 2003. The effects of different alcoholic drinks on lipids, insulin and haemostatic and inflammatory markers in older men. *Thrombosis and haemostasis*, 90(6):1080-1087.

WELLEN, K.E. & HOTAMISLIGIL, G.S. 2005. Inflammation, stress, and diabetes. *The journal of clinical investigation*, 115(5):1111-1119.

WHITNEY, E. & ROLFES, S.R. 2005. Understanding nutrition. 10th ed. CA: Thomson Wadsworth. 708 p.

WIMAN, B., ANDERSSON, T., HALLQVIST, J., REUTERWALL, C., AHLBOM, A. & DEFAIRE, U. 2000. Plasma levels of tissue plasminogen activator/ plasminogen activator inhibitor-1 and von Willebrand factor are significant risk markers for recurrent myocardial infarction in the Stockholm Heart Epidemiology Program (SHEEP) study. *Arteriosclerosis, thrombosis, and vascular biology,* 20(8):2019-2023.

WOLMARANS, P., LANGENHOVEN, M. & FABER, M. 1992. Food, facts and figures. Cape Town: Oxford University Press. 118p.

WU, H., CAI, P., CLEMENS, D.L., JERRELLS, T.R., ANSARI, G.A. & KAPHALIA, B.S. 2006. Metabolic basis of ethanol-induced cytotoxicity in recombinant HepG2 cells: role of nonoxidative metabolism. *Toxicology and applied pharmacology,* 216(2):238-247.

YARNELL, J.W.G., SWEETNAM, P.M., RUMLEY, A. & LOWE, G.D.O. 2000. Lifestyle and haemostatic risk factors for ischemic heart disease: the Caerphilly study. *Arteriosclerosis, thrombosis, and vascular biology,* 20(1):271-279.

YE, S., GREEN, F.R., SCARABIN, P.Y., NICAUD, V., BARA, L., DAWSON, S.J., HUMPHRIES, S.E., EVANS, A., LUC, G., CAMBOU, J.P., ARVEILER, D., HENNEY, A.M. & CAMBIEN, F. 1995. The 4G/5G genetic polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene is associated with differences in plasma PAI-1 activity but not with risk of myocardial infarction in the ECTIM study. *Thrombosis and haemostasis,* 74(3):837-841.

YUDKIN, J.S., STEHOUWER, C.D.A., EMEIS, J.J. & COPPACK, S.W. 1999. C-Reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arteriosclerosis, thrombosis, and vascular biology,* 19(4):972-978.

YUSUF, S., REDDY, S., ÔUNPUU, S. & ANAND, S. 2001. Global burden of cardiovascular diseases: part 1: general considerations, the epidemiologic transition, risk factors, and impact of urbanization. *Circulation,* 104(22):2746-2753.

YUSUF, S., HAWKEN, S., ÔUMPUU, S., DANS, T., AVEZUM, A., LANAS, F., MCQUEEN, M., BUDAJ, A., OAIS, P., VARIGOS, J. & LISHENG, L. 2004. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet,* 364(9438):937-952.

ZAKHARI, S. 2006. Overview: how is alcohol metabolized in the body? *Alcohol research & health,* 29(4):245-254.

ADDENDA

ADDENDUM A

PURE informed consent form

POTCHEFSTROOM CAMPUS

PURE-SA Project (Prospective Urban and Rural Epidemiology)

INFORMED CONSENT FORM (including the PRIMER-study)

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

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

I hereby also declare that I am aware that:

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

.....

(Signature of the subject)

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

Witnesses

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

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

PART 1

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

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

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

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

5.. **Aim of this project**

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

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

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

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

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

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

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

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

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

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

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

PART 2

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

1. Participation in this project is voluntary.

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

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

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

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

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

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

Dr ANNAMARIE KRUGER

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

ADDENDUM B

Quantitative food frequency questionnaire

PURE Quantitative Food Frequency Questionnaire

Subject ID **Subject Initials**

☐☐	☐☐☐	☐☐☐	☐☐	☐☐☐
Centre #	Community #	Household #	Subject #	F M L

Today's date: ☐☐☐☐ ☐☐ ☐☐
year *month* *day*

1. Name: _____
_____

2. Not applicable in South Africa

3. National identity # or equivalent _____ **N/A** ☐

4. DOB: ☐☐☐☐ ☐☐ ☐☐ **OR** Age ☐☐ years

5. Sex: ☐ Female ☐ Male

Please think carefully about the food and drink you have consumed during the ***past month*** (four weeks). I will go through a list of foods and drinks with you and I would like you to tell me:

- If you eat the food
- How the food is prepared
- How much of the food you eat at a time
- How many times a day you eat it and if you do not eat it everyday, how many times a week or a month you eat it.

To help you to describe the amount of a food you eat, I will show you pictures of different amounts of the food. Please say which picture is the closest to the amount you eat, or if it is smaller, between the sizes or bigger than the pictures.

There are no right or wrong answers.

Everything you tell me is confidential. Only your subject number appears on the form.

Is there anything you want to ask now?

Are you willing to go on with the questions?

FOOD FREQUENCY QUESTIONNAIRE

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

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

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

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

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

If yes, how much milk								

Do you put sugar on your porridge or cereal? Yes No

If yes, how much sugar							3989	
							3989	
							3989	
Samp	Bought Self ground						3250	
Samp and beans	Give ratio of samp:beans						3402 (1:1)	
Samp and peanuts	Give ratio of samp:peanuts						3250 (samp)	
Rice	White						3247	
	Brown						3315	
	Maize Rice						3250	
Pasta	Macaroni Spaghetti Other specify: _____ _____						3262	
Pizza	Home made: Specify topping _____ _____						3353 (base+ch)	

	Bought: Specify topping _____ _____						3353 (base+ch)	

You are being very helpful. Can I now ask you about meat?

CHICKEN, MEAT, FISH

How many times do you eat meat (beef, mutton, pork, chicken, fish) per week?

_____

Chicken (codes with skin)	Boiled						2926	
	Fried: in batter/crumbs Eg Kentucky						3018	
	Fried: Not coated							
	Bought: Chicken Licken						2925	
	Bought: Nando's							
	Roasted / Grilled						2925	
	Other: _____							

Do you eat chicken skin? Always | Sometimes | Never

Chicken bones stew								
Chicken feet							2997	
Chicken offal								
Red meat	How do you like meat? With fat Fat trimmed							
Red meat	Fried							
	Stewed							
	Mince with tomato and onion						2987	
	Other:							

Beef Offal	Intestines: boiled nothing added						3003	
	Stewed with vegetables							
	Liver						2920	
	Kidney						2923	
	Other: Specify _____ _____							
Goat meat	Boiled						4281	
	Stewed with vegetables							
	Grilled / Roasted						4281	

What type of vegetables is usually put into meat stews?

_____

Wors / Sausage							2931	
Bacon							2906	
Cold meats	Polony						2919	
	Ham						2967	
	Vienna						2936	
	Other: Specify _____ _____ _____							
Canned meat	Bully beef							
	Other: Specify _____ _____							

Meat pie	Beef						2939	
	Steak and kidney						2957	
	Cornish						2953	
	Chicken						2954	
	Other							
Hamburger	Bought							
Dried beans/peas/lentils	Soup						3145	
	Salad							
Soya products eg. Toppers	Brands at home now: _____ _____						3196 (Toppers)	
Pilchards in tomato/chilli/brine	Whole						3102	
	Mashed with fried onion							
Fried fish	With batter/crumbs							
	Without batter/crumbs							
Other canned fish	Tuna						3056 (oil)	
	Pickled fish							
	Other: Specify _____							
Fish cakes	Bought: Fried						3080	
	Home made with potato						3098	
Fish fingers	Bought						3081	
Eggs	Boiled/poached						2867	
	Scrambled: milk + fat							
	Fried: Fat							

Now we come to vegetables and fruit

VEGETABLES AND FRUIT

Cabbage	How do you cook cabbage?							
	Boiled, nothing added						3756	
	Boiled with potato and onion and fat							
	Fried, nothing added Fried in							
	Boiled, then fried with potato, onion							
	Other:							
	Don't know							
Spinach/morogo/ beetroot leaves other green leafy	How do you cook spinach?							
	Boiled, nothing added						3913	
	Boiled with fat added Type of fat							
	With onion, tomato, potato							
	With peanuts							
	Other:							
	Don't know							
Tomato and onion gravy	Home made with fat Type of fat							
	Without fat						3925	
	Canned						4192	
Pumpkin (yellow)	How do you cook pumpkin?							
	Boiled, nothing added						4164	
	Cooked in fat and sugar Fat							
	Boiled, little sugar and fat Fat							
	Other							
	Don't know							

Carrots	How do you cook carrots?							
	Boiled, nothing added						3757	
	Boiled, sugar and fat Fat							
	With potato and onion: Fat							
	Raw, salad						3709	
	Chakalaka							
	Other							
	Don't know							
Mealies/ Sweet corn	How do you eat mealies?							
	On cob – fat added Fat							
	On cob – no fat added						3725	
	Creamed sweet corn / canned						3726	
	Whole kernel/canned						3942	
Beetroot	Salad						3699	
	Boiled, nothing added						3698	
Potatoes	How do you cook potatoes?							
	Boiled/baked with skin						4155	
	Boiled/baked without skin						3737	
	Mashed							
	Roasted Fat							
	French fries (chips)						3740	
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked with skin						3748	
	Boiled/baked without skin						3903	
	Mashed							
	Other: _____							
	Don't know							

Salad vegetables	Mixed salad: tomato, lettuce and cucumber						3921	
	Raw tomato						3750	
	Other salad vegetables: _____ _____							
Other vegetables, specify + preparation	_____ _____ _____							
Do you like fruit?		Yes		No				
Apples							3592	
Pears							3582	
Oranges							3560	
Naartjie							3558	
Grapes							3550	
Peaches	Fresh						3565	
	Canned						3567	
Apricots	Fresh						3534	
	Canned						3535	
Mangoes							3556	
Guavas	Fresh						3551	
	Canned						3553	
Avocado							3656	
Wild fruit/berries	Specify type: _____							
Dried fruit	Types: _____							

Other fruit	_____							

If subject eats canned fruit: Do you have custard with the canned fruit? Yes 1 No 2

Custard	Home made: Milk							
	Commercial eg Ultramel						2716	

BREAD AND BREAD SPREADS

Bread / Bread rolls	White						3210	
	Brown						3211	
	Whole wheat						3212	

Do you spread anything on the bread? Always 1 Sometimes 2 Never 3

Margarine	What brand do you have at home now?							
	Don't know _____							
Peanut butter							3485	
Jam/syrup/honey							3985	
Marmite / Fray bentos / Oxo							4058	
Fish/meat paste							3109	
Cheese	Type: _____ _____							
Achaar								

Other spreads	Specify: _____							
Dumpling								
Vetkoek	White flour						3257	
	Whole wheat flour						3324	
Provita, crackers, etc							3235	
Mayonnaise / salad dressing	Mayonnaise						3488	
	Other: Specify _____							
DRINKS								
Tea	English (normal)						4038	
	Rooibos						4054	
Coffee							4037	
Sugar/cup tea or coffee	Tea:						3989	
	Coffee:						3989	
Milk/cup tea or coffee	What type of milk do you use in tea and coffee?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Whole milk powder Brand: _____						2721 (powder)	
	Low fat milk powder Brand: _____						2825 (powder)	

	Skimmed milk powder Brand: _____						2825 (powder)	
	Milk blend Brand: _____						2770 (powder)	
	Whitener: type _____ _____							
	Condensed milk						2714	
	Evaporated milk						2715	
	None							
Milk as such	What type of milk do you drink milk as such?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Condensed milk						2714	
	Sour/maas						2787	
	Other: _____ _____							
Milk drinks	Nestle: _____							
	Milo: _____							
	Flavoured milk: _____							

	Other:							
Yoghurt	Drinking yoghurt						2756	
	Thick yoghurt						2734	
	Low fat sweetened with fruit						2732	
Squash	Sweet O						4027	
	Six O							
	Oros/Lecol – with sugar						3982	
	- artificially sweetener						3990	
	KoolAid						4027	
	Other: _____ _____							
Fruit juice	Fresh/Liquifruit/Ceres						2866	
	Tropica (Dairy –fruit juice mix)						2791	
	Other: _____ _____ _____							
Fizzy drinks Coke, fanta, etc	Sweetened						3981	
	Diet							
Maueu/Motogo							4056	
Home brew								
Tlokwe							4039	
Beer							4031	
Spirits							4035	
Wine red							4033	
Wine White							4033	

Other specify	_____ _____ _____							
SNACKS AND SWEETS								
Potato crisps							3417	
Peanuts	Raw						4285	
	Roasted						3458	
Cheese curls, Niknaks, etc							3267	
Raisins							3552	
Peanuts and raisins								
Chocolates	Name: _____ _____ _____							
Candies	Sugus, gums, hard sweets, etc						4000	
Sweets	Toffees, fudge, caramels						3991	
Biscuits/cookies	Type: _____ _____ _____							
Cakes and tarts	Type: _____ _____							

	_____							
Scones								
Rusks	Type: _____ _____							
Savouries	Sausage rolls						2939	
	Samoosas: Meat filling						3355	
	Samoosas: Vegetable filling						3414	
	Biscuits eg bacon kips							
	Other specify: _____							
Jelly							3983	
Baked pudding	Type: _____							
Instant pudding	Milk type: _____							
Ice cream							3483	
Sorbet							3491	
Other specify	_____ _____ _____							

SAUCES, GRAVIES AND CONDIMENTS

Tomato sauce / Worcester sauce							3139	
Chutney							3168	
Pickles							3866	
Packet soups							3165	
Other:	_____ _____							
WILD BIRDS, ANIMALS OR INCECTS (hunted in rural areas or on farms)								
Wild fruit								
MISCELLANEOUS: Please mention any other foods used more than once/two times a week which we have talked about:								

INDIGENOUS/TRADITIONAL FOODS/PLANTS/ANIMALS

Please tell me if you use any indigenous plants OR other indigenous foods like mopani worms, locusts ect to eat

Specify								

Thank you very much for your cooperation and patience.

Good-bye!