

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Cardiovascular disease is a major risk factor for morbidity and mortality. The number of deaths caused by cardiovascular disease in developing countries is increasing and is higher, in some instances, than in many developed countries (Gaziano, 2007).

Cardiovascular disease is caused by various factors, one of which is an abnormal haemostatic process (Ajjan & Grant, 2006; Folsom, 2001; Lefevre *et al.*, 2004). Haemostasis is responsible for the formation and degradation of blood clots, which occurs as a result of injury to the vessel wall. If the formation and degradation process of the blood clots is not balanced and formed clots are not appropriately lysed, thrombosis can result (Ajjan & Grant, 2006; Folsom, 2001; Lefevre *et al.*, 2004).

One of the haemostatic factors considered to be an independent risk factor for cardiovascular disease is fibrinogen (Ajjan & Grant, 2006; Lefevre *et al.*, 2004; Kakafika *et al.*, 2007). Fibrinogen is an acute-phase protein and the precursor to the formation of a stable fibrin clot (Folsom, 2001). Fibrinogen can play a role in the development of atherosclerotic plaques by moving into the intima of the injured vessel walls, where it forms cross-linked fibrin clots (Feinbloom & Bauer, 2005). After cross-linked fibrin clots are formed in the intima, fibrin stimulates smooth muscle cell proliferation and migration, where fibrin then forms part of atherosclerotic plaques in the vasculature (Folsom, 2001; Kakafika *et al.*, 2007; Libby *et al.*, 2011). Atherosclerotic plaques have a fibrous cap which can rupture when it has a weak tensile strength (Aikawa & Libby, 2004), resulting in the activation of procoagulant material in the bloodstream which initiates the coagulation cascade (Libby *et al.*, 2011). When a blood clot or thrombus forms inside a blood vessel it has the potential to obstruct the blood flow through the system and can result in ischaemia (Libby *et al.*, 2011). Elevated fibrinogen, an important soluble coagulation factor, increases the risk of the occurrence of thrombosis (Uitte de Willige *et al.*, 2009a).

Elevated fibrinogen concentrations can be caused by demographic, environmental and genetic factors (Kakafika *et al.*, 2007). Demographic and environmental factors that can affect fibrinogen concentrations are gender, ethnicity, age, smoking, alcohol intake, psychological and social factors, infection, metabolic syndrome, hypertension, blood lipids, physical exercise, nutrition and hormone replacement therapy (Kakafika *et al.*, 2007). Heritability can have a significant influence on fibrinogen concentrations as it is reported to contribute 40-50% of the variation in fibrinogen concentrations in twin studies (De Lange *et al.*, 2001; Scott *et al.*, 2004). Genetic factors that can affect fibrinogen concentrations include various polymorphisms situated in the genes encoding the fibrinogen molecule. Fibrinogen consists of intertwining α helices of the A alpha ($A\alpha$), B beta ($B\beta$) and gamma (γ) polypeptide chains, which are each encoded by separate genes (Cooper *et al.*, 2003; Lee *et al.*, 1999).

The polypeptide fibrinogen γ chain has two different variants, termed the $\gamma A/\gamma A$ and $\gamma A/\gamma'$ (Lovely *et al.*, 2007; Uitte de Willige *et al.*, 2009a). The two variants differ regarding the amount of constituent amino acids (Lovely *et al.*, 2007; Uitte de Willige *et al.*, 2009a). The last four amino acids of the $\gamma A/\gamma A$ variant are replaced by twenty amino acids in the $\gamma A/\gamma'$ variant. Thus the $\gamma A/\gamma'$ variant has sixteen amino acids more than the $\gamma A/\gamma A$ variant (Cooper *et al.*, 2003). Approximately 8-15% of total plasma fibrinogen concentration is comprised of the $\gamma A/\gamma'$ variant (Chung & Davie, 1984; Fornace *et al.*, 1984). The presence of the fibrinogen gamma prime (γ') chain can influence the clot structure and this is relevant to cardiovascular disease because of its prothrombotic and antithrombotic properties (Uitte de Willige *et al.*, 2009a).

Many studies have investigated fibrinogen in Caucasian individuals, but only a few studies have investigated fibrinogen in non-Caucasians (Lee *et al.*, 1999; Pieters *et al.*, 2011), while no information at all is available on fibrinogen γ' concentrations in non-Caucasian individuals. The genetic background variation of different ethnic groups differs, which could explain the higher fibrinogen concentration observed in African Americans when compared with Northern Europeans (Austin *et al.*, 2000), as well as the high fibrinogen concentration observed in black South Africans (James *et al.*, 2000; Pieters *et al.*, 2011).

Fibrinogen is known to increase with age (Kamath & Lip, 2003). Therefore, in addition to expecting differences between the genetic make-up of our population and that previously reported for other populations, it will be valuable to investigate whether subjects harbouring different genotypes will have different changes in total fibrinogen and fibrinogen γ' concentrations over time. It is hypothesised that the changes over time in subjects harbouring the different polymorphisms will be more pronounced in some genotypes than others and that some polymorphisms will be sensitive to environmental changes. We decided to analyse ten single nucleotide polymorphisms (SNPs) in the fibrinogen α (FGA), fibrinogen β (FGB) and fibrinogen γ (FGG) genes. These SNPs are: FGA 2224 G>A [rs2070011], FGA 6534 A>G [rs6050], FGB 1038 G>A [rs1800791], FGB Arg448Lys [rs4220], FGB -148 C>T [rs1800787], FGB 1643 C>T [rs1800788], FGB 40 A>G [rs2227385], FGB 749 A>G [rs2227388], FGG 10034 C>T [rs2066865] and FGG 9340 T>C [rs1049636], where G is guanine, A is adenine, C is cytosine and T is thymine. In the literature, these polymorphisms have been reported to affect fibrinogen and fibrinogen γ' concentrations (Ajjan *et al.*, 2008; Feinbloom & Bauer, 2005; Grünbacher *et al.*, 2007; Kakafika *et al.*, 2007; Lane & Grant, 2000; Lim *et al.*, 2003; Mannila *et al.*, 2006). We investigated how these genotypes affected changes in total fibrinogen and fibrinogen γ' concentrations over a five-year period in black South Africans (n≈2000) participating in the 12-year follow-up Prospective Urban and Rural Epidemiology (PURE) study. We additionally determined whether possible gene-environment interactions influenced these changes over time.

1.2 AIM AND OBJECTIVES

The main aim of the proposed study was to determine the change in fibrinogen and fibrinogen γ' concentrations over a five-year period (2005-2010) in a black South African cohort (n≈2010) subdivided according to genotypes associated with fibrinogen and fibrinogen γ' concentrations, and to determine whether the observed changes were modulated by environmental factors.

Specific objectives were:

- To determine the baseline (2005) and follow-up (2010) concentrations of fibrinogen and fibrinogen γ' of a black South African population residing in the North West Province.
- To determine the genotype distributions of the FGA 2224 G>A [rs2070011], FGA 6534 A>G [rs6050], FGB 1038 G>A [rs1800791], FGB Arg448Lys [rs4220], FGB -148 C>T [rs1800787], FGB 1643 C>T [rs1800788], FGB 40 A>G [rs2227385], FGB 749 A>G [rs2227388], FGG 10034 C>T [rs2066865] and FGG 9340 T>C [rs1049636] SNPs within the black South African cohort.
- To determine the change in fibrinogen and fibrinogen γ' concentrations over the five-year period within the different genotypes, as mentioned above, of a black South African population in the North West Province.
- To assess the major lifestyle determinants of fibrinogen and fibrinogen γ' concentrations in black South Africans.
- To determine whether the hypothesised changes in the fibrinogen and fibrinogen γ' in subjects harbouring the different genotypes were modulated by the environmental factors identified.

1.3 RESEARCH TEAM

	Affiliation	Role in the study
Prof. M Pieters	Centre of Excellence of Nutrition, North-West University, Potchefstroom Campus	Supervisor of M.Sc. dissertation, guidance regarding protocol and literature review writing, statistical analysis, interpretation of results and writing up of the data.
Dr C. Nienaber-Rousseau	Centre of Excellence of Nutrition, North-West University, Potchefstroom Campus	Co-supervisor of M.Sc. dissertation, guidance regarding protocol and literature review writing, statistical analysis, interpretation of results and writing up of the data.
Mr W. Dreyer	Centre of Excellence of Nutrition, North-West University, Potchefstroom Campus	Assisted with laboratory analysis of fibrinogen and fibrinogen γ'
Ms E. Rossouw	Centre of Excellence of Nutrition, North-West University, Potchefstroom Campus	Assisted with laboratory analysis of fibrinogen and fibrinogen γ'
Ms A Jobse	Centre of Excellence of Nutrition, North-West University, Potchefstroom Campus	Full-time M.Sc. student, protocol writing, laboratory analysis of haemostatic variables, literature review and methodology writing, statistical analysis, interpretation of results and writing up of data

1.4 STRUCTURE OF THIS DISSERTATION

This dissertation is written in chapter format, presented from chapter 1 to 5. It was edited by a competent language editor and was technically edited in the style required by the North-West University. Chapter 2, which is a literature review, follows this introductory chapter. The literature review consists of an explanation of the physiological process of haemostasis, the biochemistry of fibrinogen and fibrinogen γ' and their roles in cardiovascular diseases. It also explains the effect that genetic SNPs have on fibrinogen and fibrinogen γ' concentrations. An explanation of gene-environment interactions of fibrinogen and fibrinogen γ' are also given as well as an explanation of the importance of determining the role of these factors in predicting change in the fibrinogen variables over time.

Chapter 3, which is the methodology chapter, explains how ethical approval was obtained, the method of recruitment, the characteristics of the participants, the study design, all the experimental methods including anthropometrical measurements, blood pressure measurements, adult questionnaire completion, dietary intake analysis, assessment of physical activity, blood sampling and analysis, human immunodeficiency virus testing and genetic analysis of SNPs. This chapter also includes the methodology regarding the statistical analysis employed in the study.

Chapter 4 provides all the results determined by this study. Firstly, it gives the basic descriptive characteristics of the PURE population at baseline (2005) and follow-up (2010). Differences in the fibrinogen variables associated with urbanisation and gender are also provided, as well as associations between the fibrinogen variables and environmental factors. The genotype distribution and genetic linkage disequilibrium determination of the SNPs investigated in this study follows next. Differences in the fibrinogen variables related to the different genotypes of the SNPs are then presented. This chapter also furnishes the results of the influence of gene-environment interactions on the fibrinogen variables cross-sectionally as well as how environmental or genetic factors influenced the change in the fibrinogen variables over the five years. Lastly, the chapter summarises gene-environment interactions that possibly influenced the change in the fibrinogen variables over the five-year period.

Chapter 5 discusses all the results of this study in combination with the current literature. Possible reasons for the results obtained are given. This chapter describes how urbanisation and gender influenced total fibrinogen and fibrinogen γ' on a cross-sectional level and how they affected the change in these variables over time. This is followed by a discussion of the cross-sectional association of environmental and genetic factors with the fibrinogen variables as well as with the change in concentrations over the five-year study period. The minor allele frequencies of the analysed SNPs are also compared with the literature in order to determine how the distributions in black South Africans compare with what is reported for other ethnicities. Lastly, the possible effect of gene-environment interactions on fibrinogen variables is discussed on both a cross-sectional as well as a prospective level.