THE EFFECT OF A COMBINATION OF SHORT-CHAIN FATTY ACIDS ON PLASMA FIBRINOGEN CONCENTRATIONS IN WESTERNISED BLACK MEN

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Dissertation submitted in order to meet the requirements for the degree Magister Scientiae in Dietetics in the Faculty of Health Science, Department of Human Nutrition, at the University of the Orange Free State.

November 1999

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ABBREVIATIONS

Å	Angstrom
α	Alpha
Acetyl-Coa	Acetyl coenzyme A
ACS	Acetyl-CoA synthetase
AI	Adequate Intake
AIM	Aperture Integrity Monitor
Apo (a)	Apolipoprotein A
Apo (b)	Apolipoprotein B
ATP	Adenosine-5'-phosphate
ATP II	Adult treatment panel II
β	Beta
BCG	Bromocresol-green
BMI	Body mass index
BRISK	Black population coronary risk study
°C	Degree celcius
Ca^{2+}	ionic calcium
Cat. no.	catalogue number
cfas	Calibrator for automised systems
CHD	coronary heart disease
Cl^-	ionic chlorine
cm	Centimetre
CO_2	Carbon dioxide
code no.	code number
CRP	c-reactive protein
CV	coefficient of variation
Dal	Dalton
EAR	Estimated Average Requirement
Factor VII	Proconvertin
Factor VIII	antihaemophilic factor
FDP	fibrinogen degradation products
FFA	free fatty acids
FFQ	food frequency questionnaire

FVIIc	coagulation factor VII
FVIIIc	coagulation factor VIII
g	Gram
g/L	gram per litre
H^+	ionic hydrogen
HCO_3^-	hydrogen carbonate
HDL cholesterol	high density lipoprotein
hr	Hour
IBS	Irritable bowel syndrome
IDDM	Insulin dependent diabetes mellitus
IHD	Ischaemic heart disease
K^+	Ionic potassium
Kcal	Kilocalories
Kg	Kilogram
KGM	Konjac-glucomannan
KJ	Kilojoules
L	Litre
LDL cholesterol	Low density lipoprotein cholesterol
LED	Light Energy Display
Lp (a)	Lipoprotein (a)
LPL	Lipoprotein lipase
Maximum	Maximum
MDH	Malate dehydrogenase
Med	Median
Mg	Milligram
MI	Miocardial infarction
Min	Minimum
mL	Millilitre
mmHg	Millimetre Mercury
mmol/L	Millimole per litre
MUFA	Monounsaturated fatty acids
Na^+	Ionic sodium
NAD	Nicotinamide-adenine dinucleotides

NADH	Nicotinamide-adenine dinocleotide phossphate
NCEP	National Cholesterol Education Program
NIDDM	Non-insulin dependent diabetes mellitus
Nm	Nanometer
NSP	Non-starch polysaccharide
Ox-LDL	Oxidised LDL
P	Pressure
PAGE	Polyacrylamide gel electrophoresis
PAI-1	Plasminogen activator inhibitor type 1
PAI-2	Plasminogen activator inhibitor type 2
pH	Percentage hydrogen
P/S ratio	Polyunsaturated fatty acid / saturated fatty acid ratio
PUFA	Polyunsaturated fatty acids
RBC	Red blood cell count
RE	Retinol Equivalents
Rpm	Revolutions per minute
RDA	Recommended dietary allowances
RDI's	Recommended dietary intakes
SAIMR	South African Institute for Medical Research
SANDF	South African National Defence Force
SCFA	Short-chain fatty acids
SD	Standard deviation
SFA	Saturated fatty acids
T	Time
TC	Total cholesterol
TE	Total energy
TG	Triglycerides
TP	Total protein
t-PA	Tissue-plasminogen activator
μg	Microgram
UK	United Kingdom
UL	Tolerable Upper Intake
μmol	Micromol
UOFS	University of the Orange Free State
u-PA	Urokinase type plasminogen activator

USA	United States of America
μ_T	Mass to length ratio of fibrin fibres
V	Volt
VIC	Vitamin Information Centre
VLDL	Very low density lipoprotein cholesterol
ω-6	Omega 6 polyunsaturated fatty acids
ω-3	Omega 3 polyunsaturated fatty acids
WBC	White blood cell count
WHC	Water-holding capacity
WHO	World health organisation
WHR	Waist to hip ratio
γ	Gamma

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ACKNOWLEDGEMENTS

I would like to express my gratitude and sincere appreciation to the following individuals and organisations for their much valued assistance, support and contributions to the successful completion of this study.

- Prof. Andrè Dannhauser, Head of the Department of Human Nutrition, UOFS, and study leader, for her expert guidance, encouragement and support during the execution of the study.

- Dr. Derick Veldman, Head of the Fibrinogen Unit, Technikon Free State, co-supervisor, for his much valued guidance, advice and assistance as well as the laboratory analysis and financial support.

- Quatromed who supplied and prepared the capsules used for the study is gratefully acknowledged.

- The enthusiastic study population, who followed the protocol without any complaints.

- Me Gena Joubert, Department of Biostatistics, UOFS, for her time and effort with statistical analysis.

- Personnel from the Fibrinogen Unit at the Technicon, for their assistance with the analysis of the blood samples.

- The following personnel of the School of Armor unit:

  - The Commanding Officer, Col. Andrè Retief, for making available soldiers and precious time to participate in the study.

  - The commanding officers of the Tank Regiment who were involved in the planning and execution of the study as well as in helping to keep the subjects motivated.

- Mess personnel for their patience and support.
- Medical personnel of the sickbay who helped with obtaining data.

- Financial support by the SAMHS that made this study possible.

- My husband Eli, for his love, support, encouragement and sacrifices made.

CHAPTER 1

PROBLEM STATEMENT

1.1 INTRODUCTION

Cerebrovascular disease and coronary heart disease (CHD) are some of the most important causes of morbidity and mortality amongst South Africans and also in the Western world (Bradshaw *et al*., 1995). Furthermore, the incidence of the western diseases, atherosclerosis, CHD and cerebrovascular disease, is progressively rising in black populations in South Africa (Mollentze *et al*., 1995; Kahn & Tollman, 1999). Stroke is the most rampant clinical entity of cerebrovascular disease (CVD) (Steyn *et al.*, 1992), and is an important cause of death in westernised black populations in South Africa (Joubert, 1991; Qilibash, 1995; Kahn & Tollman, 1999) as well as black populations in the USA (Iso *et al*., 1989). As the risk factor prevalence for stroke and CHD becomes altered by changes in lifestyle and diet, westernisation and migration to an urban environment, the risk of stroke and CHD is likely to rise further (Steyn *et al.,* 1991; Seedat *et al*., 1992; Bourne *et al*., 1993; Mollentze *et al*., 1995; Solomons & Gross, 1995).

Westernised black populations tend to have elevated fibrinogen concentrations (Venter *et al*., 1992; Slabber *et al*. 1997). Venter *et al.* (1992) also demonstrated that westernisation of blacks raises fibrinogen concentrations even before an increase in serum lipoproteins is observed. Raised plasma fibrinogen concentrations are accepted as an independent risk factor for stroke and CHD (Cook & Ubben, 1990; Wolf, 1994). Apart from the known coronary risk factors and raised plasma fibrinogen levels, other haemostatic risk factors, including modified network structures, factor VII, factor VIII and C-reactive protein (CRP), are also accepted as important risk factors for the development of these western diseases (Wilhelmsen *et al*., 1984; Stone & Thorp, 1985; Kannel *et al*., 1987; Yarnell *et al*., 1991; Blombäck *et al*., 1992).

The prudent low-fat, high-fibre diet is regarded as one of the controllable risk factors in the prevention of degenerative western diseases and is therefore also effective in controlling known coronary risk factors (hyperinsulinaemia, and hyperlipidaemia,

hypertension, obesity, etc.), as well as raised clotting factors (Vorster, *et al*., 1988; Hubbard, *et al*., 1994; Vorster *et al*., 1997). There is some evidence that fat intake may influence factor VII (Meade *et al*., 1986) and that fibrinogen levels may be lowered by fish oil (Oosthuizen *et al.*, 1994), alcohol (Meade and North, 1977) and soluble dietary fibre (Silvis *et al.*, 1990; Vorster *et al.,* 1997a). Veldman *et al*. (1999) also indicated beneficial effects on haemostasis through pectin supplementation. Furthermore, Venter *et al.* (1997) stated that a supplement of soluble fibre might not only improve glucose tolerance and reduce serum lipid and lipoprotein concentrations but also reduce fibrinogen concentrations. The effect of diet on haemostatic risk factors is, however, still controversial and not well established. This underlines the importance of investigating possible effects of diet in haemostasis.

The physiological effects of dietary fibre in humans are significantly influenced by the degree to which fibre is fermented in the colon (Cummings, 1982; Bourquin, *et al*., 1992). Colonic fibre fermentation results in the production of short-chain fatty acids (SCFAs) acetate, propionate and butyrate (Muir *et al*., 1995; Bugaut & Bentejac, 1993). Total SCFA production from fermentation has been found to be the greatest for the soluble fibre, oat bran (Bourquin *et al.,* 1992a). Further effects of SCFAs on lipid metabolism (Topping & Wong, 1994), haemostasis (Veldman *et al*., 1999) and factor VII activity (Marckmann & Jespersen, 1996) are also evident.

Few results, however, are found regarding the effect of SCFAs on haemostatic factors in human subjects. Veldman *et al.* (1999) indicated that acetate has a small non-significant decreasing effect on fibrinogen concentration, but found a significant difference in the characteristics of fibrin networks. The significant difference in the characteristics of fibrin networks includes a decrease in network fibrin content, which indicates that less fibrinogen is converted to fibrin. These fibrin fibres are eventually incorporated into the fibrin networks, which are believed to be less atherogenic (Veldman *et al*., 1999). From these limited observations it is evident that there is a possible association between dietary fibre or SCFAs, fibrin network architecture and some other haemostatic risk factors. This observation, however, lacks thorough investigation.

Mollentze *et al*. (1995) indicated that the black population in the Free State is already in advanced stages of urbanisation and westernisation, while Bourne *et al*. (1993) found that urbanisation of black populations in the Cape Peninsula represents a phase towards a progressively atherogenic western diet. Furthermore, Slabber *et al*. (1997) also indicated that urban African men in the Free State show a tendency towards an atherogenic westernised diet, characterised by low-fibre, high-fat intake.

The African members of the South African National Defence Force (SANDF) are exposed to a westernised lifestyle and eating habits due to their higher income, western menus and the exposure to cigarette-smoking and alcohol use. This could lead to a change in the risk profile of the members. If it is found that a supplement of SCFAs has beneficial effects on fibrinogen and other haemostatic and coronary risk factors, it can be used in the treatment and prevention of western diseases such as CHD and stroke. The health aspects associated with westernisation will increase the burden on health workers in the future, therefore this could be of great importance in decreasing future medical expenditure.

1.2 AIM

The main aim of the study is to examine the effect of a combination of SCFAs on plasma fibrinogen concentrations and some other haemostatic and coronary risk factors in westernised black men.

1.3 OBJECTIVES

The objectives of the study are to determine the effect of a combination of SCFAs, the fermentation products of oat bran (acetate:propionate:butyrate – 65:19:16) on :

- Haemostatic risk factors (plasma fibrinogen, fibrin network architecture, factor VII and factor VIII activity);
- reversible coronary risk factors (obesity, blood pressure, serum lipids);
- relevant metabolic indicators; and
- fasting acetate concentrations.

1.4 STRUCTURE OF THESIS

The first chapter of the study consists of an introduction with the motivation for and aim of the study. Chapter two is an extensive literature review on the most critical information needed to understand and interpret the study. The methodology used in the study is discussed in chapter three, and the results are given in chapter four. Chapter five presents the discussion of results, followed by conclusions and recommendations in chapter six. Examples of the questionnaires used in this study as well as some results of the dietary questionnaires are given as appendices at the end of the thesis. For a clear understanding of definitions and terms used in the study, the terminology will be defined at the beginning of chapter two.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

CHD accounts for a major proportion of deaths in most industrialised populations (Bradshaw *et al*., 1995). Control of coronary disease depends upon prevention or treatment of its known risk factors (Lewis *et al*., 1989. p. 1). Most of the known risk factors such as clotting factors and blood flow as well as lipid infiltration, which are important in the development of atherosclerosis and its clinical sequelae (Meade *et al*., 1980), could be related to lifestyle (O'Keefe *et al*., 1995).

Most of the cardiovascular disease (CVD) risk factors have been found to be related to fibrinogen (Møller & Kirstensen, 1991; Rosengren *et al*., 1990). Fibrinogen may be involved in several aspects of cardiovascular diseases, including the effects of classic risk factors, haemostatic disturbance and inflammation (Koenig *et al*., 1998). Elevated plasma levels of coagulation factor VII (FVIIc) and factor VIII (FVIIIc) have also been associated with an increased risk of CHD (Meade *et al.,* 1986).

Fibrinogen levels should be considered when evaluating the urgency for preventive measures in the candidate for cardiovascular disease and when selecting therapy to correct other risk factors related to elevated fibrinogen levels such as hypertension, dyslipidaemia or glucose intolerance (Kannel, 1997). Vorster *et al*. (1997a) reviewed the relationship between diet and haemostasis to support the hypothesis that the protective effect of diet is also mediated through the haemostatic system.

2.2 TERMINOLOGY

To understand CHD risk and other terms used in this chapter, it will be useful to provide some definitions and criteria as a framework.

Angina: Chest pain resulting from impaired blood flow to the heart (ischaemia) (Krummel, 2000, p. 558).

Atherosclerosis: Atherosclerosis is an aggregated inflammatory response to injury of the endothelial and smooth muscle cells of the arterial wall (Manson *et al*., 1996, p.32). It can also be defined as a complex process of thickening and narrowing of the arterial walls of the large- and medium-sized blood vessels caused by the accumulation of lipids, primarily oxidised cholesterol, in the intimal or inner layer in combination with connective tissue and calcification (Krummel, 2000, p. 558).

Cardiovascular disease (CVD): Any disease that causes damage to the heart or to arteries that carry blood to and from the heart (Edlin *et al*., 1998, p.520).

Coronary heart disease (CHD): CHD is a multicausal disease manifested by atherosclerosis and/or thrombosis (Hubbard *et al*., 1994), that involves the network of blood vessels surrounding and serving the heart; manifested in clinical end-points of myocardial infarction and sudden death (Krummel, 2000, p. 558).

Coronary Risk Factors (CRF): Risk is a measure of likelihood of an event occurring (Swales & de Bono, 1993, p. 1). A risk factor is a trait predicting the probability of developing this disease. Several criteria have been used as guidelines in judging whether an epidemiological association reflects a causal role of a particular risk factor. These include the strength of the association and its consistency in different studies and populations (Prevention, 1992). By these criteria, the haemostatic and other risk factors that fully qualify as causal risk factors for CHD and stroke will be discussed.

Haemostasis: The term haemostasis means the property of the blood circulation system which maintains the blood in a fluid state within the vessel walls in combination with an ability to prevent excessive blood loss when injured (Bishop *et al*., 1996, p. 719).

Myocardial infarction (MI): Death of heart tissue caused by blockage preventing the flow of blood through its coronary arteries (Williams, 1990, p.474).

Non-starch polysaccharides (NSP): All dietary fibre, except lignin, are plant polysaccharides and are therefore termed non-starch polysaccharides (WHO, 1998).

NSP can be classified by solubility in water, since water-soluble (pectin) and water-insoluble (cereal) fibres have distinct physiological effects (Slavin, 1987).

Stroke: Stroke is a localised neurological blood shortage due to a vascular lesion. Sudden loss of cerebral function with coma due to bleeding, thrombosis or embolism of a cerebral artery (Edlin *et al*., 1998, p. 297).

Thrombosis: Development of a blood clot (thrombus) that lodges in a blood vessel and cuts off the blood supply at that point (Williams, 1990, P476).

2.3 THEORETICAL MODEL

Figure 2.1 presents a theoretical model of variables indicating the relationship between dietary fibre, SCFAs, fibrinogen and related haemostatic and coronary risk factors.

2.4 CORONARY RISK FACTORS (CRF)

Atherosclerotic heart disease is a multifactorial disease. The disease and its sequelae encompass genetic factors; physiological factors, such as metabolism of the arterial wall; humoral factors, including lipid and lipoproteins and the complex mechanics of blood clotting; stress and similar psychological factors; and ecological factors which include diet and behaviours such as cigarette-smoking (Kritchevsky, 1994). A synergism is also described between these risk factors for CHD and stroke (WHO, 1990). The CRF will be discussed in terms of haemostatic risk factors, and the possible relationship between haemostatic and other coronary risk factors according to Fig 2.1.

2.4.1 Haemostatic risk factors

The relation of elevated fibrinogen levels with CHD was first voiced in the 1950's (reviewed by Ernst & Resch, 1993). During the last decade, not only raised plasma fibrinogen levels but also elevated levels of factor VII, factor VIII, fibrin network and

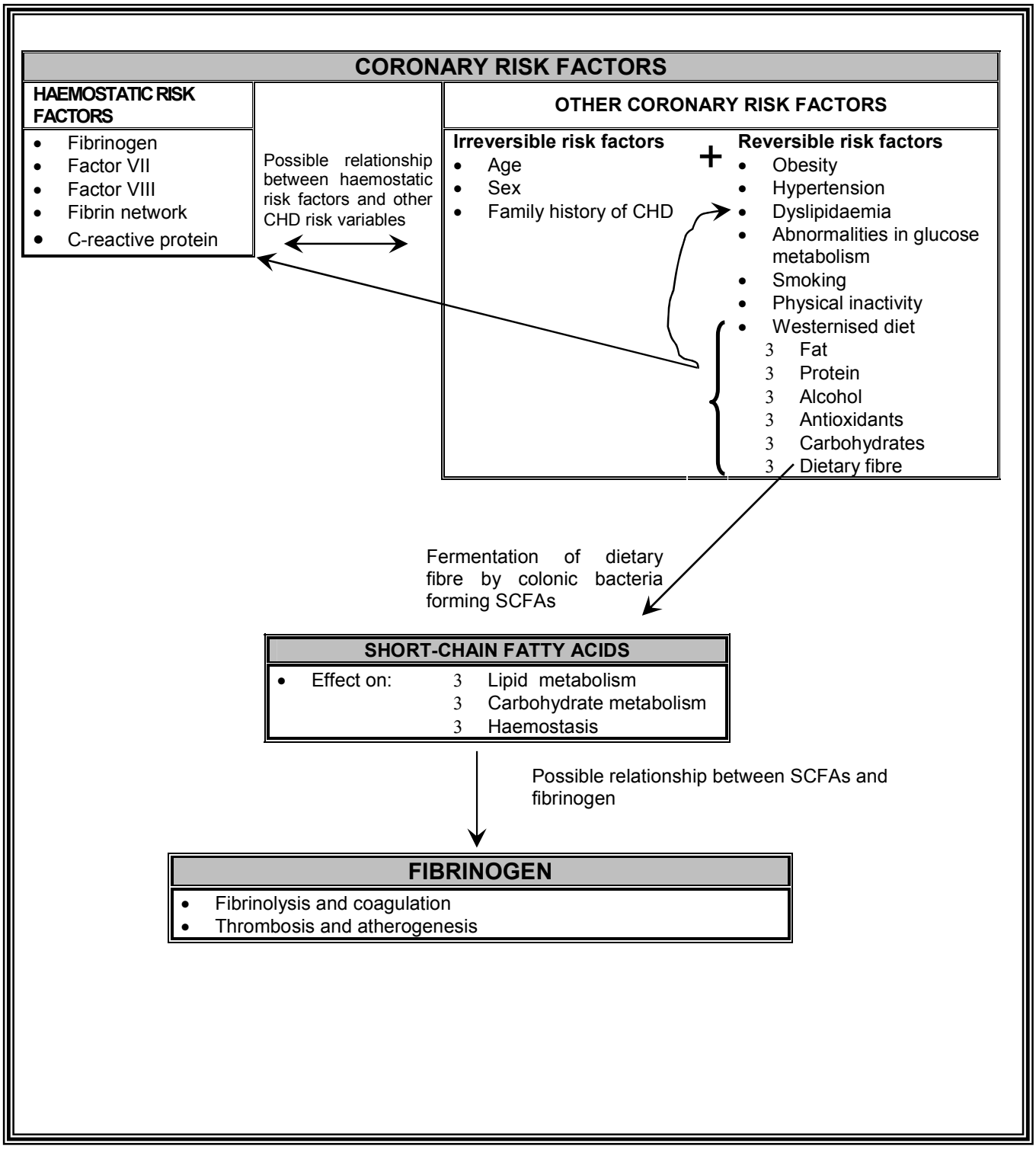


Fig. 2.1 **Theoretical model of variables, which indicates the relationship between dietary fibre, short-chain fatty acids, fibrinogen and related coronary risk factors** (Lewis *et al*., 1989, p. 7; Swales & de Bono, 1993, p. 142-143; Kritchevsky, 1994; Vorster & Venter, 1994; Anderson, 1995; Engelhardt,1995; Kannel & Wilssson, 1995; Rémésy *et al.*, 1995; Wolever, 1995; Veldman *et al.*1999).

C-reactive protein (CRP) are associated with the development of CHD, stroke, and cardiovascular mortality (Meade *et al.*, 1980; Wilhelmsen *et al*., 1984; Meade *et al.,* 1986; Kannel *et al*., 1987; Blombäck *et al.*, 1992; Haverkate *et al.*, 1997). Meade *et al.* (1986) indicated that the biochemical disturbance leading to CHD lies as much in the coagulation system as in the metabolism of cholesterol. It can be accepted that the independent association of fibrinogen, factor VII and factor VIII with cardiovascular mortality is at least as strong as the association of such deaths with blood cholesterol (Meade *et al*., 1986; Kannel*,* 1997). Fibrinogen may play an important part in the early evolution of stroke. The Framingham Heart Study indicated that, except for hypertension, fibrinogen is a strong risk factor for stroke (Kannel *et al.*, 1987). High fibrinogen levels may increase the risk of thrombus formation at an atherosclerotic plaque (Wilhelmsen *et al*., 1984) and could influence the rate of occurrence of diseases with a thrombotic component (Kannel *et al*., 1987). The above-mentioned studies as well as several other authors as summarised in Table 2.1 indicate that raised fibrinogen levels are related to the incidence of both initial and recurrent events of CHD and stroke. The possible relationship shown in Table 2.1 between haemostatic risk factors with CHD and stroke indicates the need for a thorough discussion of the haemostatic risk factors.

2.4.1.1 Fibrinogen

Plasma fibrinogen is the source of fibrin, the main protein involved in forming a thrombus (Swales & de Bono, 1993, p. 142). Fibrinogen is an acute-phase protein found in elevated concentrations in patients with inflammatory disease. Fibrinogen also plays an important role in the causal pathway for atherosclerosis or in that significant “inflammation” that accompanies asymptomatic atherosclerosis (Yarnell *et al*., 1991; Salonen *et al*., 1992; Tracy *et al.*, 1995).

Evidence from clinical and population-based studies strongly implicate fibrinogen as a major risk for atherosclerotic cardiovascular disease and stroke. These studies also describe the influence of fibrinogen (independently and in combination with other risk factors) on the occurrence of initial and recurrent cardiovascular events.

Table 2.1 Studies indicating a relationship of haemostatic risk factors with CHD and Stroke.

References:	Relationship of haemostatic risk factors with CHD and Stroke
The Framingham Study (Kannel *et al.*, 1987)	• In men, the impact of high fibrinogen levels was significant for both initial and recurrent events (CHD, stroke, cardiac failure or peripheral artery disease), adjusting only for age. After adjustment for other major cardiovascular risk factors, this effect remained evident and was statistically significant for initial and recurrent events.
The Gothenburg Study (Willhelmsen *et al.,* 1984)	• The fibrinogen level was significantly higher in subjects who had a myocardial infarction or stroke than in those without these end points. • In univariate analyses, fibrinogen was a significant risk factor for infarction and strokes, whereas the other coagulation factors were not. • In multivariate analyses, in which systolic blood pressure, smoking and serum cholesterol were controlled, only the relationship between stroke and fibrinogen remains significant.
The Northwick Park Heart Study (Meade *et al.*, 1986)	• Fibrinogen and FVIIIc activity was significantly higher in those who died of cardiovascular disease than in those who survived. • The mean entry fibrinogen level was higher for those in whom CHD developed than for those who remained CHD-free. • An increase of one standard deviation in factor VII and fibrinogen raised the risk of CHD death within 5 years of recruitment by about 55% and 67%, respectively. • The biochemical disturbance leading to CHD may lie as much in the coagulation system as in the metabolism of cholesterol.
The PROCAM Study (Heinrich *et al,* 1994)	• The independent association of fibrinogen with the risk of CHD was demonstrated even after the addition of HDL cholesterol and family history of CHD to the risk factors. • Higher FVIIc activity for all CHD events was confirmed.
The Caerphilly and Speedwell Collaborative Heart Disease Study (Yarnell *et al,* 1991)	• Mean levels of fibrinogen were higher in men who developed major CHD. • Univariate analyses show that fibrinogen, white blood cell count and viscosity are all strongly associated with the incidence of major CHD.
Lee *et al.*, 1993	• The risk of having either MI or angina increased as plasma fibrinogen concentrations increased. • Subjects with a medical history of stroke had higher plasma fibrinogen concentrations.
Fowkes, 1995	• Elevated fibrinogen levels are related to peripheral artery disease.
Cushman *et al.*, 1996	• Fibrinogen was positively related to prevalent cardiovascular disease. • High factor VIII level was associated with subsequent ischaemic events in those with pre-existing vascular disease.
Pan *et al.*, 1997	• The presence of mild and moderate carotid plaque was significantly associated with a high level of factor VIII.
Mendall *et al.*, 1996	• In patients with unstable angina and chronic CHD, CRP concentration may be a powerful predictor of subsequent cardiac events. • Strong association between CRP concentrations and CHD.
Haverkate *et al.*, 1997	• Slightly increased production of CRP is common in patients with angina and is significantly associated with increased risk of myocardial infarction and sudden cardiac death.

Table 2.1 Studies indicating a relationship of haemostatic risk factors with CHD and Stroke. (continued)

References:	Relationship of haemostatic risk factors with CHD and Stroke
Liuzzo *et al.*, 1994	• Plasma concentrations of CRP are elevated in the majority of patients with unstable angina, MI and a history of unstable angina.
Ridker *et al.*, 1997	• The baseline plasma concentration of CRP in apparently healthy men can predict the risk of first myocardial infarction and ischaemic stroke, independently of other risk factors. • The risk of arterial thrombosis associated with the level of CRP was stable over long periods and was not modified by other factors, including BMI, blood pressure, total cholesterol, HDL cholesterol, triglycerides, Lp(a) and fibrinogen.
Kuller *et al.*, 1996	• CRP is an acute-phase protein that is apparently a marker for increased risk of CHD.
Landin-Wilhelmsen *et al.,* 1997	• High fibrinogen levels were found in acromegaly (acromegaly is associated with increased morbidity and mortality from cardiovascular disease, and stroke in particular).

The studies mentioned in Table 2.1 include the Göthenburg Study (Wilhelmsen *et al.*, 1984), the Framingham Study (Kannel *et al.,* 1987), the Northwick Park Heart Study (Meade *et al.*, 1986), the PROCAM Study (Heinrich *et al.,* 1994), the Caerphilly Speedwell Study (Yarnell *et al.,* 1991) and the Leigh Study (Stone & Thorp, 1985). According to these, the most firmly established hazards associated with elevated fibrinogen levels are the risk of CHD and stroke. Elevated plasma fibrinogen concentrations are also associated with a medical history of stroke (Lee *et al*., 1993). Apart from this Wilhelmsen *et al*. (1984) and De la Serna (1994) also indicated that the higher the fibrinogen levels the greater the risk of manifestations of CHD.

Fibrinogen tends to cluster with other coronary risk factors, in particular abnormal lipid and glucose metabolism as well as hypertension and cigarette-smoking, which further enhances risk (Ko *et al*., 1997). According to Kannel (1997), it should be possible to lower fibrinogen levels by reducing weight, stopping cigarette-smoking, lowering blood pressure and altering the diet, measures that are already advocated for the prevention of cardiovascular disease.

Møller and Kirstensen (1991), emphasised that fibrinogen plays an important role in the development of atherosclerosis and the thrombotic process. If fibrinogen were accepted as an independent risk factor for cardiovascular disease, it would be of

great importance, from both a scientific and a preventative point of view, to know what factors influence fibrinogen.

2.4.1.2 Factor VII

Factor VII is one of the blood coagulation factors that forms part of the cascade eventually ending in the activation of prothrombin to thrombin and thus the conversion of fibrinogen to fibrin (Swales & de Bono, 1993, p. 143). Activated factor VII involves both the intrinsic and extrinsic pathways of coagulation, which may possibly result in hypercoagulability and increased fibrin formation (Jackson & Nemerson, 1980).

It seems that hypercoagulability, especially raised fibrinogen levels and factor VII activity, may play an important role not only in thrombosis, but also in the development of atherosclerosis and is therefore an important risk factor for CHD (Meade *et al,* 1980; Vorster *et al.,* 1988; Assmann *et al*., 1996; Buzzard *et al*., 1996). The Northwick Park Heart Study (Meade *et al*., 1986) and the PROCAM Study (Heinrich *et al*., 1994), as shown in Table 2.1, confirm that increased factor VII raises the risk of CHD. Higher levels of factor VII also tend to cluster with additional cardiovascular risk factors and is therefore more pronounced in the presence of age, obesity, high serum total, LDL (low density lipoprotein) cholesterol and serum triglycerides, low HDL (high density lipoprotein) cholesterol, smoking, a family history of MI, high fasting insulin levels and high levels of fibrinogen (Gliksman & Wilson, 1992; Cushman *et al*., 1996; Junker *et al*., 1997; Ishikawa *et al*., 1997). Akinkugbe (1972) stated that unfavourable lipid levels and factor VII must be a prerequisite risk factor for atherogenic effects in Blacks.

Positive associations have also been found in the Northwick Park Heart Survey and other studies between dietary fat intake and factor VII levels (Meade, 1986; Miller *et al*., 1991; Marckmann *et al*., 1993). It was further suggested that factor VII levels may be modifiable through lifestyle changes, such as dietary modification, weight reduction and lipid lowering in men and women (Tracy *et al.*, 1995; Cushman, *et al.*, 1996).

2.4.1.3 Factor VIII

Factor VIII is a large glycoprotein component that is synthesised by endothelial cells and megakaryocytes and that circulates in the plasma where it binds to arteries that have lost their endothelial cell linings, creating a surface to which platelets adhere (Hensyl, 1990, p.560). Factor VIII is therefore a key procoagulant enzymatic cofactor and is also, like fibrinogen, an inflammatory–responsive plasma protein (Tracy *et al.*, 1995).

Meade *et al.* (1980) found that the independent associations of factor VIII and fibrinogen with cardiovascular death were at least as strong as the association of blood cholesterol with cardiovascular death. Studies in Table 2.1 indicate that high factor VIII levels are associated with subsequent ischaemic events in those with pre-existing CHD and are therefore associated with carotid plaque (Cushman *et al*., 1996; Pan *et al.*, 1997). Factor VIII strongly correlated with other risk factors for CHD, such as glucose, insulin, LDL cholesterol, serum total cholesterol and serum triglyceride levels (Gliksman & Wilson, 1992; Cushman *et al*., 1996; Pan *et al*., 1997). Pan *et al.* (1997) reported a positive association between FVIIIc and carotid atherosclerosis. According to this evidence factor VIII is a CHD risk factor (Tracy *et al.*, 1995).

2.4.1.4 Fibrin network architecture

Fibrin is an elastic filamentous protein, derived from fibrinogen by the action of thrombin (Hensyl, 1990, p. 581). It is suspected that not only fibrinogen concentration but also the quality of fibrin networks may contribute to CHD risk (Blombäck *et al*., 1992). The mechanism by which elevated fibrinogen translates into higher incidences of atherosclerosis is not known, but there are many hypothesis regarding this. One possible mechanism may be through the modification of fibrin. Other possible mechanisms are :

- Fibrin stimulates specific cell proliferation and plaque formation. The fibrin structure serves as a network for cell migration and cleavage (Smith, 1986).

- Fibrin cleaves blood lipids, causing formation of a lipid-rich layer within the atherosclerotic plaque (Smith, 1986). This causes further uptake of LDL-C through the vascular wall (Ernst, 1992).
- Fibrin matrix packing within the endothelium may take place to such an extent that the diameter of the vessel may no longer be large enough for blood cells to move through the vessels (Ernst, 1993).
- Rupture of an atherosclerotic plaque allows blood to enter the plaque causing dissection of its structure and deposition of fibrin in the plaque (Verstraete, 1990).

When thrombin and fibrinogen interact, fibrin monomer is generated according to the relative amounts of the enzyme and the substrate (Nair *et* al., 1986). Aggregation generates the soluble intermediate fibrin monomer (Hantgan & Hermans, 1979). Polymers formed upon activation of fibrinogen by thrombin interact to form protofibrils, and the latter join into bundles of varying width. The early protofibrils have a width about double that of the fibrinogen molecule (Blombäck, 1996). The long, soluble fibrin monomers spontaneously associate laterally in a regularly staggered array to form the insoluble fibrin polymer network (Torbet, 1986). This causes a direct increase in the thickness of the fibrin fibres (Hantgan *et al*., 1985). Fibres and fibre bundles eventually interact to form the three-dimensional network structure of the matured fibrin gel (Blombäck, 1996). Clots formed at low fibrin concentrations have a larger porosity and longer fibre strands than gel structures formed under higher fibrinogen concentrations (Blombäck, 1996).

The physical and biochemical structure of the fibrin network depend upon the polymerisation conditions (reviewed by Diamond & Anand, 1993). It is known that any given network consists of a major network of thicker fibres and a minor network of thinner fibres (Nair *et al*., 1986). According to Blombäck *et al*. (1992), kinetic and modulating factors determine these gel structures. The kinetic factors will result in thicker, less porous networks with thinner fibres and a higher density of nodes. These structures are more rigid, as they impair the flow of a liquid through them. Conversely, low concentrations of kinetic factors result in porous networks with thick fibres and fewer nodes. These structures are deformable and plastic, since fluid easily escapes from them. The modulating factors affect the structures as they form

with otherwise constant kinetic factors. Such factors include proteins and ions in the direct surround of the fibrinogen molecule (Blombäck *et al*., 1992).

There is emerging evidence that the tendency to form fibrin networks with thin fibres at an early age is related to CHD (Nair & Shats, 1997). Fibrin network structure has been shown to be sensitive to a number of factors including pH, ionic strength, protein and disease states like peripheral vascular disease, hypercholesterolaemia, diabetes and CHD (Veldman *et al.*, 1997). Whilst initially acting as a scaffolding for cellular and biochemical processes, fibrin may also alter cell function and determine the progress of atherosclerosis (Shats *et al*., 1997).

2.4.1.5 C-Reactive Protein

Inflammation is an important feature of atherosclerotic lesions, and increased production of the acute-phase reactant, CRP, is associated with a poor prognosis in severe unstable angina. CRP levels are a measure of overall bodily inflammatory activity (Haverkate *et al*., 1997; Ridker, *et al*., 1997) and is undetected in healthy individuals (Lindsey, 1996, p. 183). CRP concentration is associated with raised serum fibrinogen, total cholesterol, triglyceride, glucose, diabetes mellitus, smoking, BMI and is strongly age-dependant (Heinrich *et al*., 1995; Mendall *et al*., 1996; Grau *et al*., 1996). Studies summarised in Table 2.1 indicate that the plasma concentration of CRP predicts the risk of future myocardial infarction and stroke (Kuller *et al.,* 1996; Mendall *et al*., 1996; Haverkate *et al*., 1997; Ridker, *et al*., 1997).

Haverkate *et al.* (1997) indicated that in patients with angina a slight increase in serum concentrations of CRP, even within the range previously considered to be normal, identifies individuals who have a significantly increased risk of progression to MI or sudden cardiac death.

D-dimer is a split product of cross-linked fibrin. D-dimers are found in the blood of healthy individuals, which suggests that there is a steady state of fibrin formation and dissolution even under physiological conditions. Theoretically, one would expect reduced fibrinolytic activity in patients with increased risk for atherothrombosis, and therefore reduced d-dimer concentrations, but a relationship between d-dimers,

fibrinogen, prothrombin and CRP is present. This provides evidence for the hypothesis of a chronic inflammatory state associated with atherosclerosis and an increase of both the acute-phase reactants CRP and fibrinogen (Heinrich *et al.*, 1995).

2.4.2 Possible relationship between haemostatic risk factors and other coronary risk factors

CHD is a multicausal disease manifested by atherosclerosis and/or thrombosis (Hubbard *et al.*, 1994). The Framingham Heart Study documented several parameters as independent predictors for CHD risk that can be categorised as reversible and irreversible risk factors (Anderson *et al.*, 1991; Hubbard *et al.*, 1994). It is not possible to reverse or change the irreversible risk factors (Thompson & Wilson, 1992, p. 4.1), while reversible risk factors can be changed through lifestyle changes and/or medication (Manson *et al.*, 1996, p. 293). The reversible and irreversible coronary risk factors are presented in Figure 2.2 (Kannel & Wilson, 1995; Manson *et al.*, 1996, p. 294).

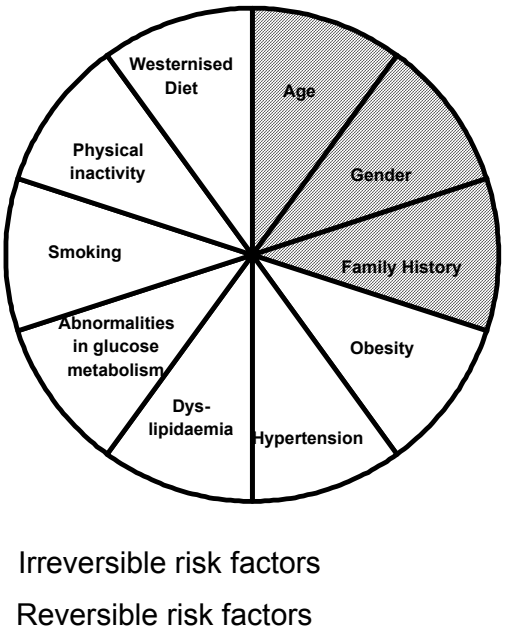


Fig. 2.2: Schematic representation of risk factors for CHD.

When fibrinogen is added to the risk profile, the individual prediction of coronary risk may be markedly improved (Kannel *et al*., 1987; Heinrich *et al*., 1994). In the Framingham Study, fibrinogen level (even in the presence of nine powerful cardiovascular risk factors) seemed to rank high among the predisposing factors for cardiovascular disease (Kannel *et al*., 1987).

Fibrinogen is related to most of the other coronary risk factors (Ernst & Resch, 1993). The independent risk factors for CHD associated with elevated fibrinogen levels are hypertension (Letcher *et al*., 1981), diabetes (Brownlee *et al.*, 1983), cigarette-smoking, obesity (Vorster *et al*., 1989), inactivity, elevated haematocrit values and dyslipidaemia (Møller & Kirstensen, 1991; Kannel, 1997). It could be possible that fibrinogen represents one mechanism whereby various other risk factors lead to CVD and this might indicate that other risk factors could mediate a fibrinogen effect (Ernst & Resch, 1993).

Wilhelmsen *et al.* (1984) found no relation between factor VIII and fibrinolytic activity with any of the conventional risk factors, while Kannel (1997) stated that an altered fibrinolytic system is likely to be an additional component of the risk factor cluster that predisposes to atherogenesis.

Besides being associated individually with CHD, the risk factors often co-exist (Steyn *et al*., 1990). The presence of several risk factors simultaneously increases the risk of CHD more than would be expected from the sum of the individual risk factors (WHO, 1990). High risk can be defined as a net of two or more CHD risk factors needing more vigorous intervention (Adult Treatment Panel II (ATPII), 1994).

Because of the high correlation of fibrinogen with the major coronary risk factors, modification of these risk factors may also provide an added benefit by reducing fibrinogen levels (Wilhelmssen *et al*., 1984; Rosengren *et al*., 1990; Møller & Kirstensen, 1991).

In the next section the relationship between the haemostatic risk factors and the irreversible and reversible coronary risk factors will be discuss. To understand this

relationship, it is also necessary to give a short background to the association between the risk factors and their role in the prevalence of CHD and stroke.

2.4.2.1 Irreversible risk factors

i) Age

Davies *et al.* (1996) reported age as a conventional coronary risk factor due to the presence of CHD with the increase in age. Coronary atherosclerosis becomes increasingly marked after the age of 20 years (Thompson & Wilson, 1992, p. 4.1). In the BRISK Study (Black Coronary Risk Factor Study) about a third of the urban black male participants of the Cape Peninsula aged 25 years and above have at least one risk factor and more than half the participants between the ages of 55 to 64 years had at least one CHD risk factor (Steyn *et al.*, 1991). According to the ATPII (1994), a rise in CHD incidence rates is observed after the age of 45 years in men. The proportion of deaths attributed to CHD increases with age, from approximately 12 percent in men aged 35 to 44 years to 27 percent in men aged 65 to 74 years (Thompson & Wilson, 1992, p. 3.4). Hypertension, dyslipidaemia, impaired glucose tolerance, physical inactivity and cigarette-smoking are all highly prevalent with aging, where there is a longer exposure to these risk factors and a diminished capacity to cope with them (Kannel & Wilson, 1995).

Stone and Thorp (1985) found a weak correlation between fibrinogen and age, while other authors found a positive association between fibrinogen and age (Fehily *et al*., 1982; Kannel *et al*., 1987; Tarallo *et al*., 1992). Meade *et al*. (1986) indicated that the association between fibrinogen was more striking in younger men. Age-related increases in CRP (Heinrich *et al*., 1995; Mendall *et al*., 1996), factor VII and factor VIII in a traditional Kitavan population were also evident (Lindeberg *et al*., 1997). The Kitavan population is a population not influenced by western dietary habits. The age-related increases in the haemostatic risk factors, fibrinogen, factor VII, factor VIII and CRP is summarised in Table 2.2.

Table 2.2 Studies indicating the relationship between haemostatic risk factors and irreversible coronary risk factors

References	Relationship between haemostatic risk factors and irreversible risk factors
Age	
Stone & Thorp, 1985	• Plasma fibrinogen correlated poorly with age.
Meade *et al.*, 1986	• The association between CHD and fibrinogen was more striking in younger men.
Kannel *et al.*, 1987	• In men, fibrinogen is related to the occurrence of cardiovascular disease at all ages, with no indication of the impact declining with age. • Below the age of 60 years, risk of CHD increased progressively with fibrinogen level in both sexes.
Tarallo *et al.*, 1992	• Fibrinogen concentrations were higher in children and adolescents than in adults aged 20-30 years. • Found an increase of fibrinogen concentration with age in adults.
Heinrich *et al.*, 1995	• CRP levels were strongly age-dependent. • Positive bivariate and multivariate regression analyses found positive correlations between plasma d-dimer concentration and age.
Mendall *et al.*, 1996	• Increasing age was associated with raised concentrations of CRP.
Lindeberg *et al.,* 1997	• Age-related increase in fibrinogen, factor VII and factor VIII in the traditional Kitavan population.
Hoffman *et al*., 1989	• Cross-sectional data indicate that higher factor VII values are found in young adults at risk for CHD.
Fehily *et al.*, 1982	• Plasma fibrinogen was positively associated with age.
Sex	
Kannel *et al.*, 1987	• Hypertension, glucose intolerance and smoking were significantly related to fibrinogen levels in both sexes. • In men, the impact of high fibrinogen levels was significant for both initial and recurrent events (CHD, stroke, cardiac failure or peripheral artery disease), adjusting only for age. • Fibrinogen values were consistently, but only slightly higher in women than in men, at all ages, especially after the menopause or with the use of oral contraceptive drugs. • Fibrinogen levels were significantly related to CHD in both men and women.
Meade *et al.*, 1986	• Men with fibrinogen levels in the upper third of the population had a three times higher CHD risk than men with fibrinogen levels in the lower third.
Lee *et al.*, 1993	• An increased risk of MI or angina associated with increased plasma fibrinogen concentrations was more striking in men.
Cushman *et al.,* 1996	• For men, the difference in fibrinogen levels was larger for non-whites versus whites and for current smokers versus non-smokers.

ii) Sex

Coronary death rates are uniformly higher in men than in women at all ages (Thompson & Wilson, 1992 p.3.5). The rates of CHD incidences are three to four times higher in men than in women during middle age, and twice as high in the elderly (ATPII, 1994). In the BRISK Study the urban male black population of the Cape Peninsula has considerable CHD risk (Steyn *et al.*, 1991). Studies summarised in Table 2.2 indicate that fibrinogen levels are consistently higher in women than in men, at all ages, but are significantly related to CHD and risk factors for CHD in both men and women, and can therefore be considered as an independent risk factor for CHD (Kannel *et al*., 1987). Furthermore, an increased risk of MI or angina associated with increased plasma fibrinogen concentrations is also more striking in men (Lee *et al*., 1993).

iii) Family history

CHD tends to cluster in families, and a positive family history of premature CHD is an important risk factor (ATPII, 1994). A positive family history of CHD indicates the prevalence of diagnosed CHD such as angina, MI, sudden death and stroke, as well as risk factors such as hypertension, hypercholesterolaemia and diabetes mellitus in mother, father, brothers, sisters, and/or children (Steyn *et al.*, 1985).

Low HDL cholesterol levels could also be due to genetic influences, where the inherited influences can be accentuated by life habits – cigarette-smoking, lack of exercise and excessive energy intake leading to obesity (ATPII,1994).

Members of families with a history of heart attacks are considered to be in the highest risk of CHD category. The risk in men with a family history of CHD is one and a half to two times as great as in men without such a history (Mahan & Arlin, 1992, p. 359). In the Framingham Study a history of CHD death in either parents was associated with a 1.3 relative risk of CHD for the children (Schildkraut *et al*., 1989).

Lee *et al*. (1993) indicated that a family history of premature heart disease is associated with increased plasma fibrinogen concentrations, where the genetic component of fibrinogen concentrations may be one contributor to the heritability of premature heart disease.

2.4.2.2 Reversible risk factors

i) Obesity

Obese patients are at increased risk of both CHD and stroke (Swales & de Bono, 1993, p. 86). Obesity commonly precedes the development of hypertension, dyslipidaemia and glucose intolerance (Thompson & Wilson, 1992, p. 9.4). In addition to predisposing to CHD by causing aggravating dyslipidaemia and hypertension, obesity defined as a body mass index (BMI) (weight in kilogram/height in meters squared) of more than 27 or an excessive accumulation of adipose fat within the abdomen is also an independent risk factor (ATPII, 1994). The Framingham Study suggested that an individual's body weight at the first examination and subsequent weight gain were both predictive of future CHD, independent of age, serum cholesterol, blood pressure, cigarette-smoking and glucose intolerance (Hubert *et al*., 1983). Heinrich *et al*. (1994) confirmed that BMI and FVIIc were associated with an approximately one and a half times higher number of events when comparing the lower with the upper tertile.

High energy intake and inactivity, leading to obesity, are associated with increased coagulation factors and decreased fibrinolytic capacity (reviewed by Vorster *et al*., 1997a). Fibrinogen levels are associated with obesity (Table 2.3) and increase with the thickness of subscapular and triceps skinfolds, chest circumference, BMI and waist to hip ratio (WHR) (Balleisen *et al.,*1985 Møller & Kirsstensen, 1996; Kannel, 1997). Table 2.3 also indicates that CRP increases significantly with BMI (Haverkate *et al*., 1997). Decreases in energy intake, weight loss and increased exercise, are associated with improvement in many haemostatic variables, including fibrinogen (Vorster *et al*., 1997a).

Table 2.3 Studies indicating a relationship between haemostatic risk factors and reversible coronary risk factors

References	Relationship between haemostatic risk factors and reversible risk factors
Obesity	
Tarallo *et al.*, 1992	• Positive relationship between fibrinogen concentrations and body weight in both males and females (10% obesity).
Kannel, 1997	• Association between fibrinogen and obesity.
Haverkate *et al.*, 1997	• CRP concentrations increased significantly with BMI.
Mendall *et al.*, 1996	• BMI was associated with raised concentrations of CRP.
Møller & Kirsten, 1991	• Fibrinogen shows a strong association with WHR in the univariate but not in the multivariate analyses.
Vorster *et al*., 1989	• Obesity or increased BMI (> 30 kg/m^2) is associated with increased plasma concentrations of fibrinogen.
Heinrich *et al*., 1994	• Confirmed that the BMI and FVIIc were associated with an approximately 1.5 times higher number of events when comparing the lower with the upper tertile.
Hypertension	
Kannel *et al.*, 1987	• Significantly related to fibrinogen in both sexes.
Willhelmsen *et al.,* 1984	• Factor VII correlated positively with systolic blood pressure. • Fibrinogen was positively related to hypertension.
Stone & Thorp, 1985	• Plasma fibrinogen correlated weakly with systolic blood pressure.
Kannel, 1997	• Association between fibrinogen and hypertension.
Lee *et al.*, 1993	• Significant association between plasma fibrinogen and hypertension.
Cushman *et al.,* 1996	• Hypertension was significantly associated with fibrinogen in men.
Heinrich *et al*., 1994	• Confirmed that the diastolic blood pressure and FVIIc were associated with an approximately 1.5 times higher number of events when comparing the lower with the upper tertile.
Raised plasma cholesterol	
Willhelmsen *et al.,* 1984	• Factor VII correlated positively with serum cholesterol concentrations. • Fibrinogen was positively related to serum cholesterol concentrations.
Stone & Thorp, 1985	• Men with high total serum cholesterol levels had a 6 times higher incidence of ischaemic events if fibrinogen levels were raised and 12 times higher if both high systolic blood pressure and elevated fibrinogen levels were present.
Yarnell *et al,* 1991	• Fibrinogen has a small positive correlation with total cholesterol.
Tarallo *et al.*, 1992	• Positive relation between fibrinogen and cholesterol.
Heinrich *et al.*, 1995	• Negative correlation was found between plasma d-dimer concentration and total cholesterol.
Cushman *et al.,* 1996	• Fibrinogen correlated with cholesterol levels in men.
Pan *et al.*, 1997	• FVIIIc was significantly associated with blood cholesterol.
Mendall *et al.*, 1996	• CRP concentration was associated with total cholesterol.

Table 2.3 (continued) **Studies indicating a relationship between haemostatic risk factors and reversible coronary risk factors**

References	Relationship between haemostatic risk factors and reversible risk factors
Raised total cholesterol (continued)	
Prisco *et al*., 1996	• Factor VII correlates with total cholesterol.
Allikmets *et al*., 1996	• Fibrinogen is associated with total cholesterol.
Kannel *et al.*, 1987	• No association between fibrinogen and serum cholesterol.
Raised LDL cholesterol	
Heinrich *et al,* 1994	• High fibrinogen added markedly to the predictive power of high LDL cholesterol. (With low fibrinogen levels, LDL cholesterol had little influence on CHD risk).
Kannel, 1997	• Association between fibrinogen and high levels of LDL cholesterol.
Heinrich *et al.*, 1995	• Negative correlation was found between plasma d-dimer concentration and LDL cholesterol.
Pepys *et al*., 1985	• CRP interacts with LDL cholesterol.
Cushman *et al.,* 1996	• Fibrinogen, factor VII and factor VIII correlated with LDL cholesterol (in men).
Mendall *et al.*, 1996	• A weak positive relation between CRP concentration and LDL cholesterol.
Halle *et al.*, 1996	• Elevated fibrinogen concentrations are associated with increased levels of circulating small, dense LDL particles. The association was independent of other risk factors associated with hyperfibrinogenaemia, such as BMI, age, insulin resistance, serum lipid concentrations and blood pressure.
Møller & Kirsten, 1991	• LDL cholesterol was independently related to fibrinogen in multivariate analyses.
Raised triglycerides	
Heinrich *et al.*, 1995	• A negative correlation was found between plasma d-dimer concentration and triglycerides.
Haverkate *et al.*, 1997	• CRP concentrations increased significantly with serum triglycerides.
Cushman *et al.,* 1996	• Factor VII correlated significantly with triglycerides.
Pan *et al.*, 1997	• FVIIIc was significantly associated with triglycerides.
Mendall *et al.*, 1996	• CRP concentration was associated with triglycerides.
Allikmets *et al*., 1996	• Fibrinogen was associated with plasma triglycerides.
Simpson *et al.*, 1983	• Patients with severe hypertriglyceridaemia have significantly higher concentrations of plasma fibrinogen than normolipidaemic comparison groups.
Halle *et al.*, 1996	• Serum triglyceride concentrations were higher in men with elevated fibrinogen levels.
Low HDL cholesterol	
Kannel, 1997	• Association between fibrinogen and low levels of HDL cholesterol.
Cushman *et al.,* 1996	• Factor VII correlates significantly with HDL cholesterol.
Halle *et al.*, 1996	• Elevated fibrinogen concentrations are associated with reduced HDL cholesterol. The relationship with HDL cholesterol was primarily determined by serum triglycerides and BMI.

Table 2.3 (continued)	Studies indicating a relationship between haemostatic risk factors and reversible coronary risk factors
References	**Relationship between haemostatic risk factors and reversible risk factors**
Raised LDL cholesterol (continued)	
Allikmets *et al*., 1996	• Fibrinogen was negatively associated with HDL cholesterol.
Mendall *et al.*, 1996	• CRP concentration was negatively associated with HDL cholesterol concentrations.
Møller & Kirsten, 1991	• HDL cholesterol is independently related to fibrinogen in multivariate analyses.
Abnormalities in glucose metabolism	
Kannel, 1997	• High levels of fibrinogen have been shown to cluster in diabetics and the metabolically linked risk factors, which also make up the insulin resistance syndrome. • Association between fibrinogen and glucose intolerance.
Kannel *et al.*, 1987	• Glucose intolerance was significantly related to fibrinogen level in both sexes.
Heinrich *et al.*, 1995	• Positive bivariate correlation was found between plasma d-dimer concentration and blood glucose.
Lee *et al.*, 1993	• Diabetic subjects had higher fibrinogen than non-diabetic subjects. Raised plasma fibrinogen concentrations may play a part in the cardiovascular complications of diabetes.
Cushman, *et al.*, 1996	• Factor VIII correlated with glucose and insulin levels and a positive relation between diabetes and factor VIII was consistent across age groups.
Allikmets *et al*., 1996	• Fibrinogen is associated with fasting glucose levels.
Mendall *et al.*, 1996	• CRP concentration was associated with raised glucose.
Smoking	
Kannel *et al.*, 1987	• Cigarette-smoking has the strongest elevating effect on fibrinogen, and therefore fibrinogen and smoking are strongly related.
Willhelmsen *et al.,* 1984	• Strong positive association between smoking and fibrinogen. • Factor VII correlated negatively with smoking.
Meade *et al.*, 1986	• The mean fibrinogen level in cigarette smokers was higher. • Much of the association between smoking and CHD may be mediated through the plasma fibrinogen level.
Heinrich *et al,* 1994	• A smoker with a high fibrinogen level had a fourfold elevated risk of CHD compared with a non-smoker with low fibrinogen.
Tarallo *et al.*, 1992	• A slight effect of smoking on fibrinogen concentration was only seen in men.
Kannel, 1997	! Association between fibrinogen and cigarette-smoking.
Heinrich *et al.*, 1995	• Negative correlations were found between plasma d-dimer concentration and smoking.
Cushman *et al.,* 1996	• Fibrinogen correlates with smoking in men.
Mendall *et al.*, 1996	• Smoking was associated with raised concentrations of CRP.

Table 2.3 (continued)	Studies indicating a relationship between haemostatic risk factors and reversible coronary risk factors
References	**Relationship between haemostatic risk factors and reversible risk factors**
Smoking (continued)	
Møller & Kirsten, 1991	• Very clear association between smoking and fibrinogen.
Kuller *et al.*, 1996	• Strong relation between levels of CRP and subsequent risk of CHD deaths among cigarette smokers.
Westernised Diet	
Tarallo *et al.*, 1992	• Diet seems to affect fibrinogen concentrations considerably.
Cushman *et al.,* 1996	• Factor VII may be modifiable through lifestyle changes. Factor VII may be reduced through lipid lowering in men.
Lindeberg *et al*., 1997	• A low-fat/high-fibre diet increases fibrinolysis. • FVIIc was decreased by low-fat/high-fibre diets.
Venter *et al*., 1991	• Animal models fed westernised high-fat diets showed that dietary fibre may lower fibrinogen levels.
Markmann *et al.*, 1994	• A low-fat/high-fibre diet may not only reduce the atherogenic but also the thrombotic tendency of an individual. • A significant decline in factor VII with a low-fat/high-fibre diet.
Miller *et al*., 1986	• Dietary fat intake has an acute influence on factor VII activity.
Fehily *et al.*, 1982	• Plasma fibrinogen was negatively associated with cereal fibre intake.
Oosthuizen *et al*., 1998	• Lecithin treatment had no independent effect on fibrinogen.

ii) Hypertension

Hypertension is a well-acknowledged CHD risk factor (Kannel & Wilson, 1995). Hypertension appears to aggravate the atherosclerotic process, possibly by weakening the artery walls at points of highest pressure, thus inviting invasion of cholesterol and other lipids (Mahan & Arlin, 1992, p. 360).

Hypertension can be defined as blood pressure of 160/95 mmHg or above, while borderline hypertension as described by the WHO is blood pressure falling between 140/90 mmHg and 160/95 mmHg (Rosssouw *et al*., 1983). Hypertension was associated with an increased risk of CHD and stroke, varying between five and thirty times, in different age and sex groups in the Framingham Study (Kannel & Wilson, 1995). According to the Framingham Study, the hypertensive patients were seven times as likely to develop stroke, six times as likely to develop congestive cardiac failure, four times as likely to develop CHD and twice as likely to develop peripheral

arterial disease as normotensive individuals (Swales & de Bono, 1993, p. 22). Hypertension is at present clinically the single most prevalent cardiovascular disease risk factor in rural as well as urban adult black South Africans (Seedat *et al*., 1992; Mollentze *et al*., 1995).

A positive relationship between hypertension and plasma fibrinogen concentrations is often present in patients with stroke and CHD, as shown in Table 2.3 by a number of authors. Hypertension was significantly associated with fibrinogen (Kannel *et al*., 1987; Lee *et al*., 1993), and Wilhelmsen *et al*. (1984), also found a correlation between factor VII and systolic blood pressure. According to Wilhelmsen *et al*. (1984), a synergistic effect appeared between fibrinogen levels and hypertension on the incidence of stroke. Blood pressure has little influence on individuals with low fibrinogen levels (Wilhelmsen *et al*., 1984). Stone and Thorp (1987), however, found a week correlation between fibrinogen and hypertension.

iii) Lipid profile

Dyslipidaemia defined as any abnormality in the lipid profile (raised total cholesterol, LDL cholesterol, triglycerides and low HDL cholesterol) (Krummel, 1996, p. 509) will be reviewed for the role it plays in CHD. According to the National Cholesterol Education Program (NCEP), increased risk of CHD is observed if two or more other risk factors are present, and if this is the case there could be a possible relationship between the risk factors present (ATPII, 1994). This clustering of risk factors is important when evaluating the risk factor profile and should therefore be taken into consideration when assessing the risk.

a. Raised plasma cholesterol

Hypercholesterolaemia refers to raised total serum cholesterol levels (Robinson *et al*., 1986, p. 532), and has been implicated in the pathogenesis of atherosclerosis (Pan *et al*., 1997). Raised serum cholesterol levels may be seen as a strong, independent risk factor for CHD (Robinson *et al*., 1986, p. 532; Lewis *et al*., 1989, p.7).

In individuals free of CHD, total cholesterol levels are classified according to Table 2.4 as “desirable blood cholesterol, high blood cholesterol” (ATPII, 1994). Within populations, blood cholesterol, particularly at high levels, is a strong predictor of CHD mortality (Krummel, 2000, p.564). Kannel (1983, p.5) indicates several factors that influence the interpretation of total serum cholesterol. The risk for CHD at any cholesterol level depends on the age, sex and presence of other risk factors such as diabetes mellitus and obesity (Kannel, 1983, p.5).

Table 2.4 Classification based on total cholesterol, LDL cholesterol, serum triglycerides and HDL cholesterol (Institute for Pathology, University of Pretoria, 1999).

	Total s-cholesterol	LDL cholesterol	S-triglycerides	HDL cholesterol
Desirable levels:	3.0 - 5.2 mmol/l	2.0 - 3.4 mmol/l	0.3 - 2.0 mmol/l	0.9 - 1.6 mmol/l
High risk levels:	>5.2 mmol/l	>3.4 mmol/l	>2.0 mmol/l	< 1.6 mmol/l

The available classification of cholesterol levels according to the Institute of Pathology in Pretoria, however, does not include variation for different age and sex groups. Patients with borderline total cholesterol levels (5.2 mmol/L to 6.2 mmol/L) who have low HDL cholesterol levels or two (or more) other risk factors or high cholesterol levels (>6.2 mmol/L) should have a lipoprotein analyses (ATP II, 1994).

Plasma fibrinogen is an independent risk factor for CHD and is positively associated with total serum cholesterol levels (Ernst & Resch, 1993). Several other studies in Table 2.3 support this positive relationship between fibrinogen and raised plasma cholesterol. Stone and Thorp (1985) found the risk of CHD to increase six times if both elevated cholesterol and fibrinogen are present. Factor VII (Wilhelmsen *et al.,* 1984; Prisco *et al*., 1996), FVIIIc (Pan *et al*., 1997) and CRP (Mendall *et al*., 1996) were also positively associated with total cholesterol.

The low serum cholesterol and LDL cholesterol levels in black populations in South Africa are in stark contrast with the high serum cholesterol levels in Whites, urban Coloureds and Indians. (Steyn *et al.*, 1987; Vermaak *et al*., 1991). Mollentze *et al*. (1995) found, however, that urbanised black populations in South African show a tendency towards total serum cholesterol levels in the higher normal range. Although black men have cholesterol levels within the normal ranges, Venter *et al.* (1992) and Slabber *et al*. (1997) reported that semi-westernised and westernised

black men had significantly higher fibrinogen levels than westernised black men. Studies indicate that male subjects with a fibrinogen level in the lower tertile of the population with high serum cholesterol and/or high blood pressure appear to have a low tendency towards cardiovascular disease. This tendency is increased six to 12 fold when a high fibrinogen level is taken into account (Stone & Thorp, 1985).

b. Raised LDL cholesterol

Low-density lipoproteins (LDL) are the primary transporters of cholesterol in the blood; consequently total cholesterol and LDL cholesterol are highly correlated (Krummel, 2000, p.566). LDL cholesterol is more closely associated with CHD than total cholesterol. The Heart Foundation of Southern Africa proposed age-related guidelines as summarised in Table 2.5 for moderate- and high-risk levels of LDL cholesterol for assessing CHD risk (Rossouw *et al*., 1988).

Table 2.5 Guide to LDL cholesterol action limits as proposed by the Heart Foundation of Southern Africa (Rossouw *et al*., 1988)

	Moderate risk	High risk
Young adults (< 30 years)	2,80 - 4,15 mmol/l	> 4,15 mmol/l
Middle-aged and older adults (> 30 years)	3,40 – 5,20 mmol/l	> 5,20 mmol/l

Table 2.4 indicates the desirable and high-risk levels of LDL cholesterol as provided by the Institute of Pathology, University of Pretoria (1999). According to ATPII (1994), the target goal of therapy for lower LDL cholesterol levels is below the cutpoints (< 4.1 mmol/L if fewer than two risk factors are present or < 3.4 mmol/L if two or more risk factors are present). The risk factors that modify the target goal for LDL cholesterol, are age (≥ 45 years old in men and ≥ 55 years old in women), a family history of premature CHD, cigarette-smoking, hypertension, low levels of HDL cholesterol, and diabetes mellitus (ATPII, 1994).

The association of CHD with increased levels of LDL cholesterol is consistent with the view that uptake of LDL (or oxidized LDL) is an important pathogenetic process that leads to the first stages of atherosclerosis (Swales & de Bono, 1992, p. 56).

During recent years there has been increasing evidence that oxidatively modified LDL (ox-LDL) may play a pivotal role in the development of atherosclerosis, because oxidative damage of LDL strongly amplifies its atherogenicity (Schalch & Weber, 1994). The size and free radical content of the LDL cholesterol particle and differences in amounts of nutrient antioxidants within the particle, as well coronary risk factors, influence the oxidation of LDL cholesterol and may exert their atherogenic effect partially through this mechanism (Table 2.6) (reviewed by Mehra *et al*., 1995). Due to oxidation, macrophages ingest modified LDL via the 'scavenger receptor'. The macrophages subsequently develop into foam cells and thus give rise to "fatty streaks", the first stage of the atherosclerotic process (Van Poppel *et al*., 1994). There is also considerable evidence that ox-LDL promotes a prothrombotic milieu. Ox-LDL stimulates expression of endothelial cell tissue factor, a known potent procoagulant. Ox-LDL also prevents endothelial cell production of prostacyclin, a platelet inhibitor, and induces plasminogen activator inhibitor 1 (PAI-1) in endothelial cells, favouring an environment of impaired thrombolysis (reviewed by Gocke & Frei, 1996).

Table 2.6 **Factors that increase susceptibility to LDL cholesterol oxidation**

Factors that increase susceptibility to LDL cholesterol oxidation
Small, dense LDL cholesterol particles
Low HDL cholesterol levels
Hypertriglyceridaemia
Cigarette-smoking
Diabetes Mellitus
Hypertension
Micronutrients
7 Low serum levels of vitamins E, C and β carotene
7 Low serum levels of selenium
7 High serum levels of iron and copper

High concentrations of fibrinogen have been found in developing lesions and an association noted between accumulation of fibrin and binding of low-density lipoproteins (Smith *et al*., 1979). High fibrinogen added markedly to the predictive power of CHD on LDL cholesterol (Halle *et al*., 1996).

With low fibrinogen levels, LDL cholesterol had little influence on CHD risk (Heinrich *et al*., 1994). The association between elevated fibrinogen levels and circulating small, dense LDL particles was independent of other risk factors associated with hyperfibrinogenaemia (Halle *et al*., 1996). These findings are summarised in Table 2.3. Cushman *et al*. (1996) also found a correlation between factor VII and factor VIII and LDL cholesterol. Pepys *et al*. (1985) indicate an association between CRP and LDL cholesterol, while Mendall *et al*. (1996) could only found a weak positive association.

c. Raised triglycerides

Controversy exists about triglycerides (TG) as an independent coronary risk factor. Because of their roles in metabolism, TG and HDL cholesterol levels are inversely related, that is, when a patient has high TG levels, the HDL cholesterol levels are usually low, and to the extent that low HDL cholesterol levels are atherogenic, elevated triglycerides likewise might be considered atherogenic (Castelli, 1992). The ATPII (1994) reviewed that triglicerydes lose their power to predict CHD if total cholesterol and HDL cholesterol are included in multivariate analyses. According to Assmann *et al.* (1996), however, they increase the risk for CHD in middle-aged men with raised TG levels, regardless of their HDL cholesterol or LDL cholesterol levels.

As indicated in Table 2.4, triglyceride levels can also be categorised as desirable and high-risk levels. Patients with borderline-high to high levels may have accompanying dyslipidaemias that increase the risk of CHD (eg. familial combined hyperlipidaemia, diabetic dyslipidaemia) (ATPII, 1994).

Raised TG levels are also associated with hypercoagulability (Simpson *et al.*, 1983), and the disturbance in the metabolsim of triglyceride-rich lipoproteins exerts prothrombotic and anti-fibrinolytic effects (Assmann *et al.*, 1996). The haemostatic risk factors fibrinogen, factor VII, factor VIII and CRP correlate positively with elevated TG, as indicated in Table 2.3 by several authors.

d. Low HDL cholesterol

HDL cholesterol is inversely associated with the risk of CHD (Rossouw *et al.*, 1985). The mechanism whereby HDL cholesterol influences CHD risk may be that HDL participates in the reverse transport of cholesterol from tissues and thereby reduces cholesterol accumulation in the arterial wall (Kannel, 1983, p.5). HDL cholesterol levels above 1 mmol/L are classified as desirable levels and a high level of HDL cholesterol (< 1.7 mmol/L) is called a "negative" risk factor (ATPII, 1994). High HDL cholesterol is considered a negative risk factor, because if a patient's HDL cholesterol is high one risk factor is subtracted from the coronary risk profile (ATPII, 1994). HDL cholesterol levels below 1 mmol/L are associated with an increased risk of CHD (Rossouw *et al*., 1988).

Freedman *et al.* (1990) found that the generalisation that mean levels of HDL cholesterol among blacks are roughly twenty percent higher than those among their white counterparts is likely not to be true at all socio-economic levels (Watkins *et al.*, 1986). HDL cholesterol reduced markedly in men who had completed high school to those who completed four or more years of college, and the highest levels were found in the low-income, unemployed group (Freedman *et al.*, 1990). Vermaak *et al*. (1991) also found that black men tend to have higher HDL levels.

Halle *et al.* (1996) and other studies (Table 2.3) showed an association between elevated fibrinogen levels, factor VII, CRP and reduced HDL cholesterol. Although Møller & Kirstensen (1991) indicated that HDL cholesterol is independently related to fibrinogen in multivariate analyses, serum triglycerides and BMI may possibly determine the relationship of fibrinogen with HDL cholesterol (Halle *et al.,* 1996).

e. Lipoprotein (a)

Lp(a) (lipoprotein a) is best described as a complex of LDL in which an additional protein component, apo (a), becomes linked to LDL via a disulphide bridge to apoprotein B-100 (Harris, 1997). Otherwise, Lp(a) has a phospholipid, triglyceride, and cholesterol content identical to LDL (Harris, 1997).

Many early studies showed that Lp(a) was a strong, independent risk factor for premature CHD, and recently Lp(a) was found to be predictive of CHD in middle-aged hyperlipidaemic men (Schaefer *et al*., 1994). High Lp(a) levels would accelerate atherosclerosis by exerting an opposing effect in promoting growth of atherosclerotic plaques (Harris, 1997). Biochemical and immunochemical studies have also established that Lp(a) is a component of the atheromatous plaque and appears to collocate with fibrin (Smith & Crosbie, 1992). Howard *et al*. (1994) found a positive correlation between fibrinogen and Lp (a). Furthermore, Slabber *et al*. (1997) showed that westernised black males in the Free State have elevated Lp (a) levels and raised fibrinogen levels.

f. Apolipoprotein A and apolipoprotein B

Several studies have suggested that plasma concentrations of apolipoprotein (apo)A-I, the major protein constituent of HDL cholesterol, and apo B, the sole protein component of LDL, are predictors of CHD (Maceijko *et al.*, 1983; Parlavecchia *et al*., 1994). Lamon-Fava *et al.* (1994) found that apo A-I concentrations are significantly higher in women than in men, and apo B concentrations were significantly lower in older than in younger men. Vermaak *et al*. (1991) showed that black South African men have lower apo B and higher apo A-I levels than their white counterparts. Diet has also been shown to affect apolipoproteins. Higher carbohydrate intake was associated with lower Apo A-I concentrations, whereas higher fat and vitamin A intake were associated with higher plasma concentrations of Apo A-I (Lamon-Fava *et al*., 1994). Apo B has been found to be positively associated with insulin (Hubbard *et al*., 1994) and FVIIc (Vaisanen e*t al*., 1995).

iv) Abnormalities in glucose metabolism

Abnormalities in carbohydrate metabolism are one of the characteristics of diabetes mellitus. Hyperglycaemia, possibly caused by insulin resistance is one of the most common causes of diabetes mellitus (Franz, 1996, p. 685). Non-insulin-dependent diabetes mellitus (NIDDM) is also associated with insulin resistance (Williams, 1990,

p.505). Epidemiological studies have shown that diabetes mellitus (Kannel *et al.*, 1986) and insulin resistance (Kannel *et al.*, 1987) are risk factors for CHD.

a. Diabetes mellitus

An abnormal glucose tolerance has an effect independent of other risk factors on the development of cardiovascular disease (Kannel *et al.*, 1987). Diabetics have a greater burden of atherogenic risk factors than non-diabetics (Lee *et al*., 1993; Kannel & Wilson, 1995), because they are more likely to be obese, hypertensive, have an adverse serum lipid profile (Swales & de Bono, 1993, p.97), and raised fibrinogen and factor VII levels (Feskens & Kromhout, 1994; Bruno *et al*., 1996). Matsuda *et al*. (1996) found that there is a thrombotic tendency or at least an imbalance between the haemostatic and thrombosis-protecting system in diabetic patients. Therefore it is evident that raised plasma fibrinogen concentrations may play a part in the cardiovascular complications of diabetes (Lee *et al*., 1993). Kannel *et al.* (1987) and other studies summarised in Table 2.3 support the fact that thrombogenic risk factors such as plasma fibrinogen, factor VIII and CRP could possibly be related to the independent effect of glucose tolerance as a cardiovascular risk factor. Cushman *et al*. (1996) indicated that the largest cross-sectional difference in factor VIII were glucose and insulin, consistent across all age groups.

b. Insulin resistance

Insulin resistance is defined as an impaired biological response to either exogenous or endogenous insulin, a common cause of NIDDM (Franz, 1996, p. 682). Insulin was found to be positively associated with blood pressure, triglycerides, total and LDL cholesterol and apo B (Hubbard *et al*., 1994). This suggests that insulin may be an important intermediate risk factor for CHD (Hubbard *et al*., 1994). Abnormalities of circulating haemostatic factors, possibly in relation with insulin resistance, may contribute to cardiovascular risk in relatives of patients with NIDDM (Mansfield *et al*., 1996). These authors also found a graded association with features of insulin resistance, which was strongest for FVIIc and weaker for fibrinogen. Insulin frequently accompanies other risk factors such as obesity, dyslipidaemia,

hypertension, decreased glucose tolerance (Ferrannini *et al*., 1987; Reaven, 1988) and fibrinogen (Abbot *et al.*, 1988; Flack & Sowers, 1991). This pattern corresponds with the insulin resistance syndrome: elevated plasma insulin concentrations, high prevalence of glucose tolerance, elevated plasma triglycerides, and low plasma HDL cholesterol (Reaven, 1988; DeFronzo & Ferrannini, 1991). High levels of fibrinogen have been shown to cluster with risk factors associated with the insulin resistance syndrome, and therefore the altered fibrinolytic system is likely to be an additional component of the risk factor cluster that predisposes to atherogenesis (Kannel, 1997). Further understanding of the insulin resistance syndrome will add another dimension to the CHD risk profile and may provide an important link between metabolic risk factors for atherosclerosis and thrombogenesis (Olivier, 1992).

v) Smoking

Smoking, particularly cigarette-smoking, is recognised as a risk factor for CHD and other atherosclerotic diseases (Holbrook *et al*., 1984; Prevention, 1992). It interacts very strongly with other important risk factors such as hypercholesterolaemia and hypertension (Swales & de Bono, 1993, p.122; Krummel, 2000, p. 569). A major reduction in CHD risk occurs even within the first year after stopping smoking. Smoking is a dangerous risk factor because it lowers HDL cholesterol, raises fibrinogen, aggregates platelets, decreases the oxygen-carrying capacity of blood, and causes release of catecholamines making the myocardium more irritable (McGill, 1979). Smoking cessation is one of the most effective ways of reducing risk of CHD and stroke (ATPII, 1994).

Cigarette-smoking has the strongest elevating effect on fibrinogen; they are therefore strongly related to each other (Willhelmsen *et al*., 1984, Meade *et al*., 1986), especially in men (Tarallo *et al*., 1992). There is already evidence that quitting smoking reduces fibrinogen levels (Kannel *et al*., 1987). It is probable that fibrinogen and smoking belong to the same chain of events leading to MI (Wilhelmsen *et al*., 1984). Several other studies summarised in Table 2.3 support the fact that cigarette-smoking has the strongest elevating effect on fibrinogen. According to Heinrich *et al*. (1994) a smoker with elevated fibrinogen levels has a four fold elevated risk of CHD, compared with a non-smoker with low fibrinogen levels. However, a negative

correlation between factor VII and smoking was found (Wilhelmsen *et al*., 1984), but CRP was found to be associated with smoking (Mendall *et al*., 1996).

vi) Physical inactivity

Physical inactivity or a low level of fitness is an independent risk factor for CHD. A sedentary person has twice the risk of developing CHD as a person who is active (Powel *et al*., 1987). A more physically active lifestyle is a useful component of a comprehensive risk reduction programme because it raises HDL cholesterol, helps lower blood pressure, improves insulin resistance, helps control obesity (Powel *et al*., 1987; Haskell *et al*., 1992), and reduces fibrinogen concentrations (Rankinen *et al*., 1994). According to Møller and Kirsten (1991) and a review by Kannel (1997), a clearly significant association between fibrinogen and physical inactivity was present in both univariate and multivariate analyses.

vii) Westernised diet

If diet was listed as a separate risk factor, it would be a risk factor for which interventions have been proven to lower CHD risk (Krummel, 2000, p. 569). According to Bourne *et al.* (1993) and Vorster *et al*. (1994), dietary patterns that meet the prudent dietary recommendations are consistent with the presence of low CHD risk factors. A high-fat, low-fibre diet reflects an increasing trend towards Westernisation and can be seen as a contributing factor to CHD risk.

Vermaak *et al*. (1991) indicated that black populations have low total cholesterol and LDL cholesterol levels and high HDL cholesterol, possibly due to ethnic (genetic) factors already present at birth. Furthermore, Vermaak *et al*. (1991) concluded that blacks are biochemically less responsive to an atherogenic diet than whites. But the incidence of CHD and cerebrovascular disease is progressively rising in the black populations and is likely to rise further as risk prevalence is altered by changes in lifestyle, Westernisation and migration to an urban and peri-urban environment (Joubert *et al*., 1989; Seedat *et al.*, 1992). The urban male black population of the Cape Peninsula already has considerable CHD risk (Steyn *et al.*, 1991) and mortality

rates from CHD among black men are similar to those reported for white men in the United States (Watkins *et al*., 1986).

Urbanisation is associated with the shift from a traditional lifestyle and eating pattern to a westernised lifestyle and diet (McMurry *et al.*, 1991; Bourne *et al.*, 1993). The traditional lifestyle and eating habits lead to a diete low in fat, and high in unrefined carbohydrates and dietary fibre. The western diet can be defined as high in animal fat and refined carbohydrates and low in dietary fibre (Walker, 1981; Bourne *et al.*, 1993; Solomons & Gross, 1995). This atherogenic dietary pattern adopted with urbanisation and improvement of socio-economic status will increase the prevalence of risk factors for CHD (Steyn *et al.*, 1991; Seedat *et al.*, 1992; Solomons & Gross, 1995). Mollentze *et al*. (1995) prove the powerful impact of urbanisation where younger black subjects in the Free-State Province, who may be adopting a western diet to a greater extent than their elders, had a higher prevalence of risk factors.

Westernisation of black and coloured men raises fibrinogen concentrations before an increase in serum lipoproteins is observed (Folsom, 1992; Venter *et al.*, 1992). Folsom (1992) also indicated that selected groups of rural blacks had levels similar to whites and significantly lower values than urbanised blacks. Diet seems to affect fibrinogen levels considerably (Tarallo *et al*., 1992). Slabber *et al.* (1997) also indicated that black men have increased fibrinogen levels accompanied by lower dietary fibre intake. Therefore it is possible, as demonstrated by Vorster *et al*. (1994), that the adoption of a western diet may result in increased plasma fibrinogen levels. A low-fat, high-fibre diet might influence not only the atherogenic but also the thrombotic tendency of an individual (Lindeberg *et al*., 1997). Fehily *et al*. (1982), found a negative association between fibrinogen and cereal fibre intake. Vorster *et al.* (1997a) support the possibility that the imprudent (high-fat, low-carbohydrate) diet could be responsible for increases of fibrinogen during Westernisation but this deserves more research. Factor VII may also be modified through lifestyle changes where a prudent diet is adopted (Cushman *et al*., 1996). Studies by Tarallo *et al*. (1992), Cushman *et al*. (1996), and Lindeberg *et al*. (1997) and several other authors as summarised in Table 2.3 support the association between dietary factors, fibrinogen levels and other haemostatic risk factors.

2.5 THE EFFECT OF DIETARY FACTORS ON CORONARY RISK FACTORS

Diet is a strong factor in the control of atherosclerosis relating to general vascular disease, CHD and stroke (Hubbard *et al.,* 1994). The dietary factors that are implicated in these processes can either be promoters of the development of CHD (cholesterol-raising and thrombogenic saturated fatty acids) or protective polyunsaturated fatty acids (PUFA) of the ω-6 and ω-3 acid series, monounsaturated fatty acids (MUFA), dietary fibre and antioxidants (Ulbricht & Southgate, 1991). Thus, it is known that diet is the predominant environmental cause of coronary atherosclerosis and that diet modification can unequivocally reduce the risk of CHD. The individual dietary factors which have been proven to lower CHD risk will be discussed according to their effect on the lipid profile as well as the haemostatic risk factors. The guidelines presented in Table 2.7 can be used as the macronutrient recommendation for the larger population for the treatment and prevention of western diseases such as CHD and stroke (ATP II, 1994). These dietary guidelines are very similar to the prudent guidelines recommended to maintain optimum health (Wolmarans *et al*., 1988).

Table 2.7 Dietary guidelines for the treatment and prevention of western diseases (Wolmarans *et al*., 1988; ATPII, 1994)

Nutrient	Recommended intake
Energy	To achieve and maintain desirable weight
Total fat	≤ 30% of total energy
Saturated fatty acids	≤ 8% of total energy
Polyunsaturated fatty acids	Up to 10% of total energy
Monounsaturated fatty acids	≥ 12 % of total energy (Up to 15% of total energy)
Cholesterol	< 300mg per day
Total Protein	12 – 20% of total energy
Plant protein	50% of total protein
Animal protein	50% of total protein
Carbohydrates	≥ 50% of total energy
Refined carbohydrates	≤ 10% of total energy
Dietary fibre	20 – 30 g per day
Alcohol	≤ 5% of total energy

2.5.1 Energy intake

Obesity often leads to raised cholesterol concentrations, LDL cholesterol, TG, lower HDL cholesterol, fibrinogen and blood pressure (ATPII, 1994; Iso *et al*., 1993). Weight reduction by decreasing energy intake reduces LDL cholesterol, triglycerides, raises HDL cholesterol and improves the haemostatic profile (Ernst *et al*., 1988; Vorster *et al*., 1997). It is therefore necessary to consume enough energy to achieve and maintain desirable body weight as described by the dietary guidelines presented in Table 2.7.

2.5.2 Dietary fat

Fat quantity, fat quality and dietary cholesterol have been investigated to see how they affect serum lipids and lipoproteins and possibly haemostasis.

2.5.2.1 Total fat intake

Total fat intakes are related to obesity, which affects many of the major risk factors for atherosclerosis (Krummel, 2000, p. 577). Total fat intake increases the relative risk of new coronary lesions (Kwiterovich, 1997). According to Blankenhorn (1990), persons consuming a diet with less than 23 percent of total energy derived from fat had a relative risk of 1 of developing new lesions, compared to 12.3 for those consuming more than 34 percent of total energy from fat. Silvis and Mollentze (1995) and Slabber *et al*. (1997) found that the total fat intake of westernised black populations in the Free State is increasing to more than the recommended thirty percent of total energy (Tabel 2.7).

2.5.2.2 Saturated fatty acids

The dietary intake of saturated fatty acids (SFA) is a major determinant of progression of CHD in symptomatic men with elevated cholesterol levels (Watts *et al.*, 1994). McMurry *et al*. (1991) indicated a dramatic increase in plasma lipids and lipoproteins if an affluent society consumed a hypercaloric diet - high in fat, energy and cholesterol and low in complex carbohydrates and fibre. This diet also leads to

increased body weight (McMurry *et al*., 1991) which is also positively and significantly associated with total cholesterol and LDL cholesterol and inversely associated with HDL cholesterol across all race-sex groups (Van Horn *et al.*, 1991).

SFA come from animal fat, dairy products and the "tropical oils" - coconut oil, palm kernel oil and palm oil this should be decreased to less than eight percent of the total energy intake of the diet (Ernst, *et al*., 1988). Table 2.8 indicates the common sources of the major dietary fatty acids as well as their effect on plasma lipids. According to Ulbricht and Southgate (1991), the atherogenic or hyperlipidaemic SFA are lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids, while the thrombogenic fatty acids are myristic, palmitic and stearic (C18:0) acids. In an *in vivo* study, Tholstrup *et al*. (1994) showed that a diet rich in stearic acid, when compared with diets rich in palmitic acid and myristic acid plus lauric acid, lowered not only serum HDL cholesterol and LDL cholesterol (Kris-Etherton & Yo, 1997) but also FVIIc. The long-chain SFA accelerate thrombus formation whereas PUFA and MUFA do not (Ulbricht & Southgate, 1991).

Table 2.8 Common sources and effect on plasma lipids of major dietary fatty acids (Kris-Etherton *et al*., 1988; Oosthuizen *et al*., 1994; Krummel, 1996, p. 51)

Fatty acids		Common sources	Major effect on plasma lipids
Saturated			
C12:0	Lauric	Coconut, palm kernel oil	Increases total, LDL & HDL cholesterol
C14:0	Myristic	Coconut, butterfat	Increases total, LDL & HDL cholesterol
C16:0	Palmic	Palm-oil, animal fat	Increases total, LDL & HDL cholesterol
C18:0	Stearic	Cocoa butter, animal fat	No effect on blood lipoproteins (neutral)
Unsaturated			
Monounsaturated			
C18:1	Oleic	Olive oil, rapeseed oil, canola oil	Decreases total and LDL cholesterol and triglyceride, with no effect on HDL cholesterol.
Polyunsaturated			
ω-6			
C18:2	Linoleic	Most vegetable oils, esp. safflower, corn, soybean, cottonseed and sunflower oil	Decreases total, LDL & HDL cholesterol.
ω-3			
C20:5 C22:6	Eicosapentaenoic Docosahexaenoic	Atlantic and king mackerel, Atlantic and Pacific herring (sardines), lake trout, chinook salmon, albacore tuna, Atlantic and sockeye salmon, bluefish, pink and chum salmon, Atlantic halibut, coho salmon, marine lipids (cod liver oil) and ω-3 fatty acid supplements	Decreases fibrinogen, triglycerides, variable LDL cholesterol and HDL cholesterol effects.

2.5.2.3. Polyunsaturated fatty acids

PUFA can be divided into ω-6 fatty acids commonly found in vegetable seeds oil and ω-3 fatty acids commonly found in fish and fish oils as shown in Table 2.8 and they should not exceed ten percent of the total energy intake (Kris-Etherton *et al*., 1988). The long-chain unsaturated ω-6 and ω-3 fatty acids are thought to be anti-atherogenic and anti-thrombogenic (Ulbricht & Southgate, 1991).

If carbohydrate is replaced by linoleic acid, the predominant dietary ω-6 PUFA LDL cholesterol is lowered and HDL cholesterol is raised. In a low-fat diet (≤ 30% of energy), replacing SFA with high PUFA intakes (up to 10% of energy) lowers total cholesterol, LDL- and HDL cholesterol (Nydahl, 1994). SFA should be partly replaced by ω-6 PUFA (Ulbricht & Southgate, 1991) because the elimination of SFA is twice as effective in lowering serum cholesterol as in increasing PUFA (Kris-Etherton *et al*., 1988). The clinical effect of low-fat diets that lower HDL cholesterol is still open to debate. It is believed that HDL cholesterol lowered by diet does not carry the risk of low HDL cholesterol before intervention, especially since the rest of the lipoprotein profile will be favourably altered by diet (NIH Concensus, 1993).

The main ω-3 fatty acids, eicosapentaenoic and docosahexaenoic, present in fish oil, lower total cholesterol, very low density lipoprotein (VLDL) cholesterol, LDL cholesterol, HDL cholesterol, triglyceride levels and hypertension (Gibson, 1988). Fish intake affects clotting factors and fibrinolytic enzymes and therefore also leads to a reduction in fibrinogen (Saynor & Gillot, 1988; Oosthuizen *et al*., 1994), and factor VIII (Iso *et al*., 1993), which is noteworthy in view of the association between plasma fibrinogen and CHD (Kannel *et al*., 1987).

2.5.2.4. Monounsaturated fatty acids

MUFA are commonly found in sunflower oil, safflower oil and most prevalent in olive oil as oleic acid (Kris-Etherton *et al*., 1988). The common sources, chemical structure as well as effect on dietary lipids are shown in Table 2.8. Replacing SFA with MUFA lowers serum cholesterol levels, LDL cholesterol, and triglyceride to

about the same extent as PUFA. The effects of MUFA on HDL cholesterol depend on the total fat content of the diet. When both MUFA and total fat intakes are high, HDL cholesterol does not change or increases slightly compared to a lower fat diet. At the recommended fat levels (≤ 30% of energy from total fat and up to 15% from MUFA), HDL cholesterol levels are decreased (Krummel, 2000, p. 577).

Oakley *et al.* (1998) indicated a rapid increase in FVIIc with a diet rich in oleic acid, while FVIIc declines after a low-fat meal. This suggests that postprandial activation of factor VII occurs rapidly after a fat-rich meal.

In conclusion Miller (1998), however, indicated that total detary fat intake is more important than dietary fat composition for factor VII.

2.5.2.5 Trans fatty acids

Trans fatty acids are formed when vegetable oils are hydrogenated to convert them from a liquid to a semisolid state that more closely resembles animal fat to either increase consumer acceptance or decrease the fat's susceptibility to oxidation (Laine *et al*., 1982). Eliadic acid, the trans isomer of oleic acid is hypercholesterolaemic when compared to PUFA, but hypocholesterolaemic when compared to myristic and lauric acid (Kris-Etherton & Yo, 1997). Trans fatty acids also raise LDL cholesterol and lower HDL cholesterol (Schaefer *et al*., 1995). Because saturated fats increase LDL cholesterol but do not decrease HDL cholesterol, trans fatty acids can produce a greater increase in the ratio of LDL to HDL cholesterol (Kwiterovich, 1997).

2.5.2.6 Dietary cholesterol

Dietary cholesterol raises total cholesterol and LDL cholesterol, but to a lesser extent than SFAs (Denke *et al*., 1994). In South African and American dietary guidelines it is advised to restrict dietary cholesterol intake to less than 300 mg per day (Truswell, 1987; Diet Consensus Panel, 1989). Sources of dietary cholesterol include the flesh of animal meats, egg yolks, organ meats (liver, brain, and sweetbreads) and some shellfish (shrimp) (ATPII, 1994). Although shellfish contain more cholesterol than

most meats, they are low in saturated fat and can be consumed within the recommended cholesterol intake (ATPII, 1994).

The Framingham Study indicated no significant relationship between dietary cholesterol levels and plasma cholesterol levels (Dawber *et al*., 1982). However, metabolic ward and controlled studies have demonstrated that an increase in dietary cholesterol as the only dietary variable, taken either as crystalline cholesterol, egg yolk or whole egg, raises serum total cholesterol (Beynen & Katan, 1989).

In addition to the effect of dietary cholesterol alone on serum lipids, dietary SFA and cholesterol have a synergistic effect on LDL cholesterol (which is associated with CHD risk) (Kris-Etherton *et al*., 1988). Furthermore, the intake of cholesterol has been positively related to the risk of CHD after adjusting for other risk factors such as age, blood pressure, serum cholesterol and cigarette-smoking (Krummel, 1996, p.530).

2.5.3 Protein intake

Dietary protein is considered to be of major importance in the aetiology of hypercholesterolaemia and atherosclerosis (Kris-Etherton *et al*., 1988). Americans consume approximately 16 percent of their energy from protein while the coloured population of the Cape Peninsula consume approximately 15 percent of their total energy from protein (Langenhoven *et al*., 1988). Furthermore, the black populations in South Africa consume between 13 to 16 percent of their total energy from protein (Wolmarans *et al*., 1988; ATPII, 1994). Total protein intake of approximately 15 percent of total energy intake, with animal and plant protein contributing 50 percent of the total protein respectively, is recommended as goal for good nutrition in preventing western diseases (Table 2.7) (Wolmarans *et al*., 1988; ATPII, 1994).

The replacement of animal protein with vegetable protein in the diet has been suggested to reduce the diet-linked atherogenic risk (Pietinen & Huttunen, 1987). Pietinen and Huttunen (1987) also indicated that strict vegetarians have lower serum cholesterol than lactovegetarians and non-vegetarians. The HDL cholesterol levels are parallel, with total cholesterol being lowest among vegetarians and highest

among omnivores. Although these differences are probably mostly related to the amount and type of fat, the effect of the type of protein cannot be excluded (Pietinen & Huttunen, 1987).

According to Kris-Etherton *et al*. (1988), animal protein is positively, and plant protein (eg. soy protein) is negatively associated with CHD. As reviewed by Hubbard *et al*. (1994) soy protein lowers serum lipid levels by means of several mechanisms in animals, and dietary protein from animal protein has been found to be related to hyperinsulinemia. Williams *et al*. (1986) also reported a significant positive correlation between consumption of animal protein and serum triglycerides, while plant protein was negatively associated with serum triglycerides. Although studies suggest that animal protein may be positively related to CHD risk factors and mortality, it is important to note that animal and SFA increase concomitantly in the diet, thereby making it difficult to examine their independent effects (Kris-Etherton *et al*, 1988).

2.5.4 Alcohol

Epidemiologic data indicate a protective effect of alcohol in moderation for CHD (Jooste *et al.*, 1988; Kannel & Wilson, 1995). This benefit applies only with moderate intakes and is not seen with alcohol abuse. According to Jooste *et al.* (1988), heavy drinking in men is associated with a cluster of adverse coronary risk factors such as hypertension, raised serum total cholesterol level, smoking and BMI. Light drinking, described as less than five percent of the total energy (Table 2.7), is associated with a more favourable coronary risk factor profile, and lowers fibrinogen levels in both smokers and non-smokers (Meade *et al.*, 1987). A linear multiple regression analyses showed a significant negative effect of alcohol in both sexes (Tarallo *et al.*, 1992), while Møller & Kirsten (1991) could not found any correlation between alcohol consumption and fibrinogen levels.

2.5.5 Antioxidants

Oxidation of LDL cholesterol (*see 2.4.2.2(iii)(b)(i)*) contributes to atherogenesis by a number of mechanisms, and antioxidants may act as anti-atherogens (Gocke & Frei,

1996). Three vitamins (C and E, and β-carotene), at physiological levels, have antioxidant roles in the body (Krummel, 2000, p. 579). Vitamin E and β-carotene are fat-soluble and can function as free radical scavengers to decrease the initiation and propagation of fatty acid oxidation (Sies & Stahl, 1995). Although Vitamin C is water-soluble and is not found in appreciable concentrations in LDL, it too has a strong antioxidant capacity and may function to regenerate or spare vitamin E (Rimm & Stampfer, 1997).

Vitamin E is the strongest evidence for cardiovascular benefit (Rexrode & Manson, 1996) as it is predominantly carried in LDL and is particularly effective in protecting LDL from oxidation. Each LDL particle appears to contain about six molecules of vitamin E (Kwiterovich, 1997). Lipid-standardised plasma levels of vitamin E strongly and inversely correlated with cross-cultural CHD mortality in sixteen European populations (Kwiterovich, 1997). The correlation coefficient was significant for lipid-standardised vitamin E plasma levels, and the correlation between plasma levels of vitamin E and CHD was greater than those for traditional risk factors, such as blood cholesterol and blood pressure levels (Kwiterovich, 1997).

Evidence from observational studies for vitamin C and clinical trials for β-carotene does not support a cardiovascular benefit (Rimm & Stampfer, 1997). However, Pandey *et al.* (1995) support the hypothesis that vitamin C and β-carotene are associated with lower risk of death from CHD. Vitamin C may protect against CHD through an effect on haemostatic factors at least partly through the response to infection (Pandey *et al*., 1995). Vitamin C was inversely related to haemostatic factors fibrinogen and FVIIc and CRP. An increase in dietary vitamin C of 60 mg per day (about one orange) was associated with a decrease in fibrinogen concentrations equivalent to a decline of approximately ten percent in risk of CHD (Khaw & Woodhouse, 1995).

The essential trace mineral, selenium, allows the enzyme glutathione peroxidase to destroy hydrogen peroxides, providing the basis for its well-known antioxidant properties (van Poppel *et al*., 1994). Low selenium levels are associated with an elevated risk of cardiovascular disease and death, but whether selenium has an

effect on LDL cholesterol oxidation has not yet been established (van Poppel *et al*., 1994; Kwiterovech, 1997).

Flavonoids found in tea, vegetables, fruit and red wine also have antioxidant properties (Kwiterovech, 1997). Flavonoids inhibit oxidation and cytotoxity of LDL, and affect haemostasis *in vitro* (Hertog *et al*., 1997).

2.5.6 Carbohydrate intake

The morbidity and mortality of CHD are the lowest in populations with a relatively high intake of carbohydrates accompanied by a low fat and cholesterol intake (Olivier, 1982). Low levels of total cholesterol, LDL cholesterol and HDL cholesterol are associated with a dietary intake of 50 to 65 percent of carbohydrates (Hallfrisch *et al*., 1988). Although high-carbohydrate, low-fat diets are associated with lower HDL cholesterol levels, the ratio of HDL to LDL cholesterol and to total serum cholesterol remains constant or increases slightly (Pietinen & Huttunen, 1987).

According to the ATP II (1994), carbohydrates should provide $\geq$ 55 percent of the total energy and can be divided into refined carbohydrates or simple sugars (monosaccharides and disaccharides) and complex carbohydrates (polysaccharides) (ATPII, 1994).

2.5.6.1 Added sugar

Available evidence indicate that increased consumption of simple carbohydrates may result in a greater plasma triglyceride response than that observed for complex carbohydrates (Kris-Etherton *et al*., 1988; Frayn & Kingman, 1995). According to the recommendations by the WHO (1990), large intake (more than 10 to 16 percent) of refined carbohydrates could be disadvantageous in that free sugars in the diet displace other energy sources such as the unrefined carbohydrates. Other reasons for limiting intakes of refined carbohydrates have been cited, including concerns about obesity and thereby, diabetes and cardiovascular disease (WHO, 1990).

2.5.6.2 Dietary fibre

Several studies indicated a positive association between the intake of dietary fibre according to the prudent guidelines and a low incidence of CHD. Westernised men and women may find it equally difficult to implement and sustain dietary behaviour recommended for the prevention of chronic disease (Posner *et al.*, 1995). An extensive investigation in the role of dietary fibre in the treatment and prevention of CHD and the possibility of supplementing a diet with the fermentation products of dietary fibre will elucidate this problem.

2.6 DIETARY FIBRE

Recommended dietary behaviour for the prevention of chronic diseases includes a recommendation to increase intake of dietary fibre. To understand the role of dietary fibre in the treatment and prevention of CHD, the fibre hypothesis as well as the classification, physical properties and therapeutic effects of dietary fibre as shown in Fig 2.1 will be discussed.

2.6.1 The fibre hypothesis

Rimm *et al.* (1996) suggest that fibre, independent of fat intake, is an important dietary component for the prevention of CHD. This relationship led to the development of a "Fibre Hypothesis" first described by Denis Burkitt and Hugh Trowell in the early 1970's (Burkitt & Trowell, 1986). The "Fibre Hypothesis" suggests:

- A diet low in fibre either causes or predisposes individuals to certain diseases.
- A diet rich in fibre helps protect against certain diseases preventable in the affluent Western communities (Burkitt & Trowell, 1986).

The dietary fibre hypothesis focussed on many comparatively neglected or unexplored aspects of nutritional physiology, especially in relation to the colon and its metabolic function (Jenkins *et al.*, 1997). During their transit through the alimentary tract, dietary fibres have ample opportunity to interact with the substrates,

effectors, and products of digestion as well as a variety of other substances progressing toward absorption or evacuation (reviewed by Deskens, 1996, p.42). Several diet-related diseases such as gastrointestinal diseases (appendicitis, diverticular disease and colon cancer), weight management, hypertension, lipid and carbohydrate metabolism and CHD, are associated with a low fibre intake (Miettinen, 1987; Wahlqvist, 1987; Walker, 1993).

The prudent guidelines and many other health organisations recommend a total dietary fibre intake of between 20 and 30 grams (Slavin, 1987; Edlin *et* al., 1998, p.94) (Table 2.7). On the basis of our current understanding, it is apparent that Western diets do not contain enough fibre; average daily fibre intake ussually falls well below the recommended level of 20 and 30 grams (Burkitt, 1982; Butrum *et al*., 1988).

2.6.2 Classification, chemistry and sources of dietary fibre

Dietary fibre is generally defined as plant material, mainly derived from plant cell walls, that is resistant to digestion by human gastrointestinal enzymes (Hunt *et al*., 1993). Food chemists prefer to define fibre as "lignin and non-starch polysaccharides (NSP)", where NSP includes cellulose, hemicellulose, pectin, gums and mucilages, found in food (Hunt *et al*., 1993). All dietary fibres, except for lignin, are plant polysaccharides and are therefore termed NSP (WHO, 1998). It has been suggested that the use of the term 'dietary fibre' be gradually phased out and replaced by NSP (British Nutrition Foundation, 1990). While there is general agreement that the NSP are the principal part of dietary fibre, there is currently no consensus as to whether other components should be included in this term (WHO, 1998). NSP are not hydrolysed by small-intestinal enzymes and are a suitable substrate for the bacterial production of short chain fatty acids (SCFAs) in the large bowel, together with incompletely digested starch, lactose and proteins (MacFarlane *et al*., 1986; Flemming & Floch, 1986). The fermentability of NSP is related to important clinical effects (Mortensen & Nordgaard-Anderson, 1993).

NSP are commonly classified by their water solubility to understand their mechanical and physiological effects (Hunt *et al*., 1993). Therefore NSP can be categorised as

soluble and insoluble fibre (Ettinger, 2000, p.41). Water-insoluble fibre includes lignin, cellulose, and many hemicelluloses, present principally in wheat, most grain products, and vegetables. Insoluble fibre shortens bowel transit time, increases faecal bulk, renders faeces softer, and delays glucose absorption and starch hydrolysis (Walker, 1993). Soluble fibre includes pectin, gums, certain hemicelloluses, and storage polysaccharides. Fruit, oats, barley, and legumes contain more soluble fibre than do other foods (Hunt *et al*., 1993; Walker, 1993). Oat bran and dried beans are especially rich sources (Walker, 1993). Soluble fibre tends to increase intestinal transit time, delays gastric emptying, slows glucose absorption and lowers serum cholesterol levels. Soluble fibre is almost completely fermented in the colon to SCFAs (Walker, 1993). The fibre in fruits, vegetables, and grains is never exclusively insoluble or soluble. The insoluble and soluble components of dietary fibre, their function and sources are summarised in Table 2.9.

Table 2.9 Components of NSP (*Adapted from Procter & Gamble, 1991)*

Fibre component and type	Function	Source
Cellulose (*insoluble*)	Basic structural material of cell walls	Whole wheat flour Wheat bran Peels of apples and pears Vegetables
Lignin (*insoluble*)	Along with cellulose, forms the woody cell walls of plants	Mature vegetables Cereal grains Wheat Fruits with edible seeds, such as strawberries
Hemicellulose (*insoluble and soluble*)	Surrounds skeletal material of cell walls and acts as cement between them	Wheat bran Whole wheat
Pectin (*soluble*)	Binds adjacent cell walls and holds water in networks	Apples Bananas Citrus fruit Strawberries Carrots
Gum (*soluble*)	Gelatinous exudate from stems or seeds	Oat bran Legumes Guar Barley
Mucilage (*soluble*)	Viscous water-holding substance, similar to gum	Seeds Seaweeds Psyllium

The most concentrated sources of dietary fibre are whole grains, especially wheat bran. Because of the higher water content, fruits and vegetables provide less NSP than the drier grains and cereals per gram of ingested material (Slavin, 1987).

Legumes and oats, are a concentrated source of water-soluble fibre (Slavin, 1987). The effect of cooking on fibre content of foods is unclear; several studies suggest that there is little difference between cooked and uncooked fruits and vegetables (Slavin, 1987). Table 2.10 gives a range of fibre content for some foods.

Table 2.10 Dietary fibre content of foods in commonly served portions (*Adapted from Ettinger, 2000, p. 40)*

Food Group	< 1 g	1-1.9 g	2-2.9 g	3-3.9 g	4-4.9 g	5-5.9 g	> 6 g
Breads (1slice)	Bagel White French	Whole wheat	Bran muffin (1)	NA	NA	NA	NA
Cereals (28 g)	Rice Crispies Special K Corn flakes	Oatmeal Nutri-Grain	Shredded Wheat	Honey Bran	Raisin Bran	Corn Bran	All Bran
Pasta (1 cup)	NA	Macaroni Spaghetti	NA	Whole-wheat Spaghetti	NA	NA	NA
Rice (½ cup)	White	Brown	NA	NA	NA	NA	NA
Legumes (½ cup) cooked	NA	NA	NA	Lentils	Lima beans Dried beans	NA	Kidney beans Baked Beans
Vegetables (½ cup unless stated)	Cucumber Letuce (1 cup) Green Pepper	Asparagus Green beans Cabbage Cauliflower Potato w/out Skin (1) Celery	Broccoli Brussels sprouts Carrots Corn Potato w/skin (1)	Peas	NA	NA	NA
Fruits (1 medium fruit unless stated)	Grapes (20) Watermelon (1 cup)	Apricots (3) Grapefruit (½) Peach w/skin Pineapple (½ cup)	Apple, w/out skin Banana Orange	Apple w/skin Pear w/skin Raspberries (½ cup)	NA	NA	NA

NA – Not Applicable

2.6.3 Physical properties of NSP

The physical properties of NSP may help describe the mechanism of action of fibre (Therapeutic, 1993, p.10). Simply characterising NSP as soluble or insoluble in water is not sufficient to explain their physiological effects. Instead, properties such as viscosity, water-holding capacity, bile acid-binding ability, particle size and microbial degradation are better predictors of physiological function (Schneeman & Tietyen, 1994). Table 2.11 highlights the nutritional and physiological implications of properties discussed in this section.

Table 2.11 Nutritional and physiological importance of physicochemical properties of dietary fibre (*Adapted from Schneeman & Tietyen, 1994*)

Property	Physiological alconsequences	Types of fibre	Nutritional implication
Microbial degradation	Breakdown of polysaccharide structure in the large intestine Production of SCFAs and other microbial metabolites Growth of microflora	Polysaccharides Extent of microbial action dependent on solubility	Faecal bulking of fibre dependent on extent of microbial degradation Increase in stool weight due to residual polysaccharides and/or increase in microbial cells Provision of energy by SCFAs to cells and possible metabolic effects Reduction in pH of colon contents
Water-holding capacity (WHC)	Swelling with water in the gut contents Increased viscosity of gastrointestinal contents Influence on the microbial breakdown on fibre	Pectins Gums β-glucans Some hemicelluloses	Increased viscosity slowing gastric emptying and the digestion and absorption of nutrients Increased viscosity interfering with mixing in intestinal contents High solubility allowing greater microbial degradation
Absorption/binding of organic molecules	Interaction with bile acids and digestive enzymes in the intestine	Pectins Gums Lignin Nonpurified fibre sources (e.g., cereal brans, legumes)	Increased faecal excretion of bile acids Slower rate of digestion in the small intestine
Particle size	Determination of surface area exposed Degree of cell wall disruption due to grinding	Primarily important for nonpurified fibre sources	Increasing surface area and disrupting cell walls enhancing exposure to microbial action and digestive enzymes

2.6.3.1 Viscosity

Viscosity or gel-forming capacity is related to a fibre's ability to absorb water and form a gelatinous mass. Soluble fibres form gels, adding viscosity to the contents of the gastrointestinal tract (Eastwood & Passmore, 1983). Gels may also provide "lubrication" to the stool (Therapeutic, 1993, p.10).

2.6.3.2 Water-holding capacity

Water-holding capacity (WHC) measures the ability of the fibre to hold water and is related to solubility of the polysaccharide (Schneeman & Tietyen, 1994). The polysaccharide constituents of dietary fibre are strongly hydrophilic. Water is held on

the hydrophilic sites of the fibre itself or within void spaces in the molecular structure (Hunt *et al*., 1993). As indicated in Table 2.11, the WHC of NSP plays a role to increase viscosity for slowing down gastric emptying and the digestion and absorption of nutrients (Schneeman & Tietyen, 1994). The high solubility due to the WHC of NSP allows greater microbial degradation (Schneeman & Tietyen, 1994).

2.6.3.2 Binding ability

Certain fibres like wheat bran, guar gum, konjac mannan (a glucomannan), chitosan, and isolated lignin have been shown to bind bile acids in the small intestine (Hunt *et al*., 1993). Sources of NSP that bind bile acids and increase their excretion may increase the turnover of cholesterol and contribute to the ability of certain fibres to lower plasma cholesterol. Although this interaction does not appear to be sufficient to explain the hypocholesterolaemic effects of fibre completely, it has important consequences for lipid utilisation (Schneeman & Tietyen, 1994) as summarised in Table 2.11.

2.6.3.4 Bulking ability

Insoluble fibres, such as cellulose and lignin, are mostly unfermentable by colonic microflora and increase faecal bulk by their particle formation and water-holding capacity (Hunt *et al*., 1993). Wheat bran is among the best bulking agents (Hunt *et al*., 1993). Soluble fibres cause additional bulking because of bacterial proliferation in the colon (Eastwood & Passmore, 1983).

2.6.3.5 Particle size

In fibre preparations, such as cereal brans, grinding can vary particle size, which disrupts the cell wall structure (Schneeman & Tietyen, 1994). Several investigators have observed that coarsely ground wheat bran is more effective in increasing stool weight and lowering intracolonic pressure than finely ground wheat bran (Eastwood & Brydon, 1985).

2.6.3.6 Microbial degradation

Most of the fibre survives passage through the small bowel but in the large intestine it is metabolised by the microflora. Since this process is anaerobic and fibre is largely carbohydrate, it is better called fermentation. As a result of fermentation, SCFAs such as acetic, propionic and butyric acids are produced and these are rapidly absorbed in the fermentation process (Cummings, 1987). Soluble fibres are fermented to a much greater extent by colonic bacteria than insoluble fibres. Research suggests that fermentation, particularly of soluble fibres, may be an important factor in some of the physiological effects caused by fibre (Edwards, 1987). Table 2.11 summarises the nutritional and physical effects of NSP due to microbial degradation, such as faecal bulking, increase in stool weight, provision of energy by SCFAs to cells and reduction in pH of colon contents.

2.6.4 Effects of NSP

Several clinical and experimental studies have been conducted in human patients and in animal models to demonstrate the need for fibre in the diet. Soluble fibres have been demonstrated to have effects on carbohydrate and lipid metabolism, while insoluble fibres are largely responsible for increasing the bulk of the faeces but have little metabolic effect (Silvis, 1992). Furthermore, it can be concluded from Table 2.11 that certain sources of NSP have the potential to be essential for normal gastrointestinal function, lowering plasma cholesterol, and blunting the glycaemic response and insulin release (Schneeman & Tietyen, 1994).

2.6.4.1 Diseases of the colon

Some diseases of the colon appear to be favourably affected by increased levels of NSP, namely constipation, diarrhoea, irritable bowel syndrome (IBS), diverticulitis, and colon cancer. Mechanical effects, those caused by the physical passage of fibre in the gastrointestinal tract, are primarily colon specific and are generally produced by both soluble and insoluble fibres (Cummings, 1987; Therapeutic, 1993, p.10; Williams, 1994, p. 42).

2.6.4.2 Obesity

High carbohydrate foods promote satiety in the short term (WHO, 1998). As fat is stored more efficiently than excess carbohydrate, use of high carbohydrate foods is likely to reduce the risk of obesity in the long term (WHO, 1998). NSP may be useful as an adjunct to hypocaloric diets in the treatment of obesity (Blundell & Burley, 1987; Stevens, 1988). There are several hypotheses to account for this effect: a diet high in fibre increases chewing time, affects insulin release during gastrointestinal transit, and may result in an increase in faecal energy loss (Rosner, 1987; Fordyce-Baum *et al.*, 1989).

2.6.4.3 Carbohydrate metabolism

NSP used in amounts unlikely to obtain from Western foods can lower blood glucose and alter hormonal profiles (especially insulin and gastrointestinal peptides) which may influence carbohydrate metabolism (Wahlqvist, 1987). High-carbohydrate, high-fibre diets are an increasingly integral part of diabetes therapy (Anderson, 1982). Water-soluble fibres, primarily pectins and gums, exert a hypoglycaemic effect by delaying gastric emptying, shortening intestinal transit, and reducing glucose absorption (Topping, 1991; Ettinger, 2000, p. 41). Fibre rich diets or diets supplemented with gel-forming, soluble fibre, have decreased or obviated the need for insulin or oral hypoglycaemic agents in patients with insulin-independent diabetes (Hunt *et al*., 1993).

2.6.4.4 Cardiovascular disease

Rimm *et al.* (1996) suggest that fibre, independent of fat intake and other risk factors, is an important dietary component for the prevention of coronary disease (Bolton-Smith *et al*., 1992; Pietinen *et al.*, 1996). NSP, especially soluble fibre, also influence the risk factors for CHD. Soluble fibres, like oat bran and fibre from beans, significantly reduce serum total and LDL cholesterol (Jenkins *et al*., 1993; ATPII, 1944; Glore *et al*., 1994) but have no consistent effect on HDL cholesterol and triglycerides (Miettinen, 1987;

Hunninghake *et al*., 1994). However, Singh *et al*. (1992) found that soluble fibre could cause a reduction in triglycerides and an increase in HDL cholesterol. The possible mechanisms for NSP to decrease blood lipids may relate to soluble NSP to 1) bind to bile acids and interfere with fat absorption, 2) increase faecal excretion of bile acids, affecting hepatic secretion of lipoproteins, and 3) fermentation by colonic bacteria, thus forming gases and SCFAs such as propionate and inhibiting hepatic cholesterol synthesis (Kritchevsky, 1988). In relation to soluble fibre, acetate has also been found to reduce cholesterol by reducing FFA concentrations (Wolever *et al.* 1989).

2.6.4.5 Haemostatic risk factors

Evidence is accumulating that a "prudent diet", especially one involving an increased intake of NSP, assures protection against hypercoagulability (Vorster *et al*., 1988). Supplementing a Westernised diet fed to baboons with konjac-glucomannan (KGM) lowered fibrinogen concentrations (Vorster *et al*., 1985). Djousse *et al.* (1998) reported that higher fibre intake is inversely associated with (PAI-1), but not with fibrinogen concentrations. Fehily *et al*. (1982) provided epidemiological evidence that high cereal fibre intakes correlated negatively with plasma fibrinogen. However, in a follow-up study, Fehily *et al*. (1986) could not demonstrate decreases in fibrinogen when healthy subjects increased their cereal-fibre (insoluble fibre) intake.

Several studies show that high-fibre diets have beneficial effects on coagulation and fibrinolysis. Low-fibre intakes are associated with impaired fibrinolysis and this situation may be rectified by increasing fibre intake (Vorster *et al*., 1997; Vorster *et al*., 1997a). The additional beneficial effect of soluble fibre on fibrinolysis is the lowering of PAI-1 levels (Venter *et al*., 1997). Veldman *et al*. (1999) found a decrease in fibrinolytic rate in pectin-supplemented subjects. This could be ascribed directly to the decrease in fibrin network content. The significant decrease in fibrin network content also indicates that less fibrinogen is converted to fibrin, which eventually is incorporated into the fibrin networks. These structures are believed to be less atherogenic (Veldman *et al.*, 1999).

Simpson *et al.* (1982) could not demonstrate a change in fibrinogen level with an increase in NSP intake in diabetic patients. But they found that coagulant activities of factors VII decreased in the higher-fibre intake in NIDDM patients, and factor VIII antigen decreased in patients with insulin-dependent diabetes mellitus (IDDM). Marckmann and Jespersen (1996) also found that oat bran has a positive effect on FVIIc and that FVIIc can be lowered 5-25% when a western diet is changed to a low-fat/high-fibre diet.

If fibre intake influences plasma fibrinogen levels, it is probably due to the soluble NSP components (Vorster *et al.*, 1997). It is evident though from these limited observations that there is a possible association between NSP and fibrinogen levels and other haemostatic risk factors. This observation, however, lacks thorough investigation.

2.6.5 Production of SCFAs

NSP, defined as undigestible complex carbohydrates, remain in the ileum but are partly hydrolysed by bacteria in the colon (Hubbard *et al*., 1994). Much of the research on the potential benefits of NSP consumption on human health has focused on the actions of fibre in the large intestine. These actions depend largely on the extent to which fibre is fermented by the residing population of anaerobic bacteria and on the physical characteristics (for example, WHC) of unfermented fibrous material (Bourquin *et al*., 1993). The main substrates for the colonic fermentation in healthy individuals are NSP (cellulose, hemicellulose and pectin) (Barry *et al*., 1995; Mortensen & Clausen, 1996). Bacterial fermentation of one gram of monosaccharide yields approximately 10 mmol of organic acid (Scheppach *et al*., 1992). Colonic fermentation leads to the production of SCFAs, certain gases (carbon dioxide, methane, hydrogen) and microbial cell mass (Bourquin *et al*., 1992). Furthermore, total SCFA production from fermentation is the greatest for oat bran (Bourquin *et al*., 1992a). Anderson (1995) also supports this in demonstrating that serum acetate levels produced from oat bran peak twice as high as those observed with intake of beans or wheat bran. The SCFAs vary widely in their relative proportions, depending upon the fibre source in the diet (Bugaut & Bentéjac,

1993). Table 2.12 indicates the SCFA molar percents from 24-hour fermentation of dietary fibres in *in vitro* incubation systems inoculated with fresh human faecal flora. After absorption, each of the primary SCFAs produced (acetate, propionate and butyrate) is metabolised by the body (Bourquin *et al*., 1992), and many biological effects of

Table 2.12 SCFA molar percents from 24-hr fermentation of dietary fibre *in vitro* (Bugaut & Bentéjac, 1993)

Substrate	Acetate	Propionate	Butyrate
Pectin	81	11	8
Gum arabic	68	23	9
Oat bran[a]	65	19	16
Wheat bran[a]	63	16	21
Cellulose[b]	53	21	26

[a] α-cellulose and hemicelluloses are 7% and 19% dry total dietary fibre, respectively, in oat bran, and 19% and 38%, respectively, in wheat bran.

[b] 48-hr fermentation.

SCFAs have been reported. The SCFAs that escape colonic metabolisation enter the hepatic portal blood, where their concentration varies over a wide range, depending on production rates, therefore on the diet (Cheng *et al.*, 1987). SCFAs influence carbohydrate and lipid metabolism (Wolever *et al*., 1991), and therefore may contribute to the protective effect of NSP against degenerative western diseases (Burkitt & Trowell, 1986). SCFAs also contribute to the energy needs of the body, and play a potential role in the protection against development of colonic disorders (Mortensen & Clausen, 1996).

2.7 SHORT-CHAIN FATTY ACIDS

2.7.1 Introduction

Fermentation in the human colon may affect metabolic events beyond the large intestine through the production of SCFAs (Venter & Vorster, 1989). Forthcoming investigation on the effect of fibre and diet on general health parameters (cholesterol,

diabetes regulation, gallstone formation, etc.) might benefit from considering that dietary components escaping small intestinal absorption is digested by fermentation and that some of its effects may be related to specific metabolites formed in the colon (Mortensen *et al*., 1992). To understand the role of SCFAs as a possible mechanism through which NSP influence the risk markers of CHD, it is important to give an overview on the absorption and metabolism of the SCFAs.

2.7.2 The absorption and metabolism of SCFAs

2.7.2.1 Introduction

SCFAs can be described as saturated unbranched alkyl monocarboxylic acids of 2-4 carbon atoms as shown in Table 2.13 (Wrong, 1995, p.2). SCFAs are biochemically more closely related to carbohydrates than to fats, some of them are not constituents of natural fats, and they are not "fatty", as the layman envisages the term, as they are completely miscible with water (Wrong, 1995, p.2). The three major SCFAs (acetate, propionate and butyrate) are moderately strong acids, with p*K* values of about 4.8. Intestinal contents are more alkaline than this, so SCFAs are predominantly present within the intestine as negatively charged *anions*, and not as free *acids* (Wrong, 1995, p. 2), and create a slightly acidic pH level (Flemming *et al*., 1992). In all regions of the colon, acetate, propionate, and butyrate account for 90-95 percent of total SCFAs, with acetate being the principal anion (Mortensen & Clausen, 1996). Serum acetate is derived primarily from colonic fermentation, serum butyrate primarily from endogenous fatty acid metabolism, and serum propionate from both exogenous and endogenous sources (Wolever *et al*., 1997). SCFAs in human faeces, following consumption of different defined polysaccharides, have been measured on the average, in the molar ratio of acetate:propionate:butyrate of 53:27:20 (Savage, 1986; Bugaut & Bentejac, 1993). The specific SCFA molar percents from different dietary fibres are presented in Table 2.12. Levels of all three SCFAs varied significantly during the day, tending to decrease after breakfast and increase transiently after lunch and dinner. Both time of

day and glucose tolerance status affect serum SCFA levels in non-diabetic humans (Wolever *et al*., 1997).

Table 2.13 Short-chain fatty acids (Mortensen & Clausen, 1996)

Chemical formula	Trivial Name
CH_3-COOH	Acetate
CH_3-CH_2-COOH	Propionate
CH_3-$(CH_2)_2$-COOH	Butyrate

SCFAs produced in the large intestine in substantial amounts are absorbed and subsequently utilised by the animal as substrate of energy metabolism (Engelhardt, 1995, p. 149). According to Mortensen and Clausen (1996), an increase of SCFA absorption is equal to 150-360Kcal/day of metabolised energy. Cummings *et al.* (1989) concluded that in people on Western diets, SCFAs absorbed from the gut were equivalent to three to nine percent of the total energy requirement. SCFAs, especially butyrate, are metabolised to different extents in the mucosa of the large intestine (Engelhardt, 1995, p. 150).

2.7.2.2 Absorption

SCFAs *in vivo* appear to be absorbed rapidly and nearly completely. Clearance rates of SCFAs have generally been observed to increase with chain length, even though there are differences between rates in the distal and proximal colon (Bugaut & Bentejac, 1993; Engelhardt, 1995, p.150). The past decade has emphasised the mechanism for SCFA absorption and the results have supported transcellular absorption as a major pathway (Mortensen & Clausen, 1996). A suggested model for transcellular transport can be either through paracellular or cellular transport (Engelhardt, 1995, p.155; Mortensen & Clausen, 1996). This also accounts for the large number of observations describing the dependence of SCFA absorption rates on luminal pH and Pco_2, as well as on fluxes of water, protons, and inorganic ions (Cl^-, HCO_3^-, Na^+ and K^+) through the colonic mucosa.

Paracellular transport is defined as a non-active transport that should depend on the transepithelial difference. However, the paracellular pathway is not a major site for SCFA absorption (Mortensen & Clausen, 1996). The possible mechanisms of cellular uptake can be through passive diffusion or facilitated diffussion (Mortensen & Clausen, 1996). The transmural movement of SCFAs is a concentration-dependent, passive diffusion process, whereby SCFAs, at least in part, are transported in the protonated form. Hydrogen ions, which are needed for SCFA protonation because 99 percent of SCFAs (pKa = 4.8) are in the ionised form at the colonic pH, may be available from Na^+ -H^+ exchange and from hydration of luminal CO_2 to HCO_3^- and H^+ (Bugaut & Bentejac, 1993). A relationship between SCFA absorption and bicarbonate secretion has frequently been observed (Engelhardt, 1995, p.157). SCFAs may also be transported in the ionised form via an SCFA-HCO_3^- exchange mechanism, the facilitated diffusion process (Bugaut & Bentejac, 1993; Mortensen & Clausen, 1996). The precise mechanism for the absorptive process of SCFAs remains, however, undefined (Bugaut & Bentejac, 1993; Mortensen & Clausen, 1996).

2.7.2.3 Metabolism

Several studies have shown that SCFAs, once taken up, were metabolised at a high rate inside the caecal and colonic mucosal cells (Bugaut & Bentejac, 1993). The SCFAs that escape colonic metabolisation enter the hepatic portal blood, where their concentration varies over a wide range, depending on intestinal production rates and therefore on the diet. The relative proportions of the three major acids in the portal blood reflect the relative proportions of those found in the intestinal contents (Bugaut & Bentejac, 1993). After absorption, each of the primary SCFAs produced is metabolised differently by the body.

i) Acetate Metabolism

Acetate largely bypasses colonic and liver metabolism but is metabolised by peripheral tissue (i.e. muscle) (Cummings *et al*, 1987a). The first step in acetate metabolism is its activation to acetyl-CoA by the enzyme acetyl-CoA synthetase, which is variably distributed in the cytosol and mitochondria of many tissues (Ballard, 1972; Wolever, 1995, p. 484).

ii) Propionate metabolism

Propionate is utilised primarily by the liver where it is used as substrate for gluconeogenesis (Bugaut & Bentejac, 1993), and it has also been suggested as a potential modulator for cholesterol synthesis (Chen *et al.*, 1984). Under normal conditions, propionate is totally taken up by the liver (Rémésy *et al*., 1995, p.177). This process is certainly favoured by facilitated diffusion, which is efficient even in the presence of relatively low concentrations of propionate (Rémésy *et al*., 1995, p.177). Propionate metabolism increases the requirements for vitamin B12, which could be critical with NSPs such as pectin, which may interfere with vitamin B12 reabsorption during its enterohepatic cycle (Cullen & Oace, 1989).

iii) Butyrate metabolism

Hepatic uptake of butyrate is considered practically total under any physiological conditions. Butyrate is a preferred energy source for coloncytes and thus is extensively metabolised by the colon (Roediger, 1982). Butyrate uptake could be facilitated by the presence of a butyrate-binding protein in the cytosol (Rémésy *et al*., 1995, p.177). Butyrate is exclusively metabolised in the mitochondria (carnitine-independent source of acetyl-CoA) and it is a potentially ketogenic substrate during the postabsorptive period. Butyrate activation is probably mediated by medium-chain acyl-CoA synthetase(s) (Rémésy *et al*., 1995, p.177). Furthermore, butyrate leads to ketone body production and was used as an important respiratory fuel in preference to acetate, propionate, and

even to glutamine, glucose and ketone bodies (Bugaut & Bentejac, 1993). High concentrations of butyrate inhibit propionate utilisation (Demignè *et al*., 1986), thus butyrate probably thwarts some of the inhibitory effects of propionate.

In conclusion, the major site of SCFA metabolism is in the liver, where propionate and butyrate are almost entirely taken up, but the percentage of acetate uptake is lower. However, due to its higher concentration in the portal vein, acetate uptake generally exceeds that of propionate and butyrate (Rémésy *et al*., 1995, p.171).

2.7.3 Effects of SCFAs on lipid metabolism

Water-soluble fibres such as psyllium, pectin, guar gum and oat gum have potent hypocholesterolaemic effects. Early research led to the suggestion that SCFAs might contribute to the hypocholesterolaemic effects of NSP (Anderson & Chen, 1979). There are two popular hypotheses concerning mechanisms for hypocholesterolaemia: 1) soluble fibres bind bile acids in the intestine, alter lipid and bile-acid absorption and increase faecal loss of bile-acids; and 2) soluble fibres are fermented in the colon to SCFAs, which are absorbed into the portal vein and attenuate hepatic cholesterol synthesis (Anderson & Siesel, 1990). This would then cause the liver to convert more cholesterol to bile acids, thereby reducing body cholesterol (Bugaut & Bentejac, 1993). The liver is the principal site of propionate metabolism and cholesterol synthesis.
Topping *et al.* (1988) reported that methylcellulose, a modified polysaccharide resistant to microbial metabolism, which therefore is not fermented in the colon to SCFAs, had no effect on plasma cholesterol, hepatic cholesterol synthesis, and faecal excretion of bile acids. Moreover, Ide *et al.* (1991) showed that guar gum hydrolysate had the same decreasing effects on cholesterol levels as did intact guar gum. A tenfold increase of caecal SCFAs contents was observed with both kinds of substrates. Veldman *et al.* (1997) found that pectin supplementation caused significant decreases in total cholesterol, LDL cholesterol, apo A, apo B and Lp(a). Such results suggest that the action of absorbed SCFAs on hepatic and peripheral metabolism of cholesterol may be

an alternative mechanism for the effect of fermentable carbohydrates on body cholesterol concentrations (Propionate and cholesterol haemostasis in animals, 1987).

It has been suggested that propionic acid and possibly even acetate may reduce plasma cholesterol levels (Bridges *et al*., 1992; reviewed by Jenkins *et al*., 1997). Wolever *et al.* (1989) suggested that acetate may reduce cholesterol synthesis by reducing circulating free fatty acids (FFA). Veldman *et al.* (1999) found that both acetate and pectin supplementation decreased FFA significantly. If cholesterol synthesis is decreased through a decrease in FFA, it is evident that both acetate and pectin could decrease total cholesterol by the same mechanism. Strong associations between changes in levels of serum LDL cholesterol and changes in levels of serum acetate also support the hypothesis that changes in SCFAs contribute to the hypocholesterolaemic effects of soluble fibres (Anderson, 1995, p. 575). Wolever *et al.* (1995), however, found that colonic acetate is incorporated into plasma lipids and that propionate resulting from colonic fermentation inhibits this process.

Venter *et al.* (1990) administered 7.5g of propionate daily in capsule form to healthy subjects for seven weeks. Propionate use did not affect serum cholesterol levels but significantly increased HDL cholesterol by 11 percent compared to control changes. Todesco *et al.* (1991) showed a significant difference in serum lipids in subjects who consumed bread containing 9.9g of propionate per day or white bread without propionate. Total cholesterol, LDL cholesterol and HDL cholesterol tended to be lower while triglycerides tended to be higher after propionate compared to white bread (Todesco *et al*., 1991).

2.7.4 Effects of SCFAs on carbohydrate metabolism

Insulin resistance, glucose intolerance and CHD partly arise from raised plasma FFA (Kaplan, 1989). Acetate may indirectly influence glucose, since oral (Crouse *et al*., 1968) and rectal (Wolever *et al*., 1991) acetate promptly reduce FFA levels in serum.

Physiological increase in FFA concentrations in the serum have been shown to reduce glucose utilisation in humans (Jenkins *et al*., 1990).

Glucose taken up by the liver can be disposed of by three routes: conversion to glycogen, the glycolytic pathway, or the pentose phosphate pathway. There is evidence that acetate, in the presence of insulin, enhances the activity of the pentose phosphate pathway (Wolever, 1995, p. 484). Acetate, despite reducing glucose flux through the glycolytic pathway, may increase net glucose utilisation by the liver, by increasing flux through the pentose phosphate pathway (Wolever, 1995, p.484).

Propionate and other fatty acids with an odd number of carbon atoms are gluconeogenic substrates (Wolever, 1995, p. 485). Wolever *et al.* (1991) showed a significant increase in blood glucose values after propionate administration. Propionate decreases glucose production from lactate in isolated hepatocytes (Anderson & Bridges, 1984). It should be noted that, under the experimental conditions used, the reduction in the rate of glucose production from lactate induced by propionate was almost equal to the rate of gluconeogenesis from propionate itself (Anderson & Bridges, 1984). Dietary propionate has been shown to reduce fasting blood glucose levels and maximum serum insulin increments (Venter *et al*., 1990) which could be consistent with reduced glucose production or enhanced utilisation (Wolever, 1995, p. 484).

2.7.5 Effect of SCFAs on haemostasis

Fehily *et al*. (1982) provided epidemiological evidence that high cereal-fibre intakes correlated negatively with plasma fibrinogen. However, in a follow-up study, Fehily *et al*. (1986) could not demonstrate a decrease in fibrinogen when healthy subjects increased their cereal-fibre intake. Morris (1994) indicated that men with a high intake of cereal-fibre have a low incidence of thrombosis. The author suggested a possible direct effect on haemostasis. Soluble NSP could have additional beneficial effects on fibrinolysis by lowering PAI-1 levels (Venter *et al*., 1997). Veldman *et al.* (1997) also indicated that the intake of pectin has beneficial effects on haemostasis by influencing

the fibrin network architecture. The effect of NSP on fibrinogen and other haemostatic variables has been discussed previously (2.6.1.3 (v)).

Vorster *et al.* (1988) suggested that the production of SCFAs, which are quickly absorbed and transported to the liver, is one of the possible mechanisms whereby NSP may influence the synthesis of the coagulation factors. Veldman *et al*. (1997) reported that pectin intake of 15g/day significantly changes the characteristics of fibrin networks in hypercholesterolaemic subjects. The networks were more permeable and had lower tensile strength and are believed to be less atherogenic. Veldman *et al.* (1999) strongly suggest that the effect of pectin on network architecture could partially be mediated by acetate. From results of *in vitro* studies, it seems highly possible that acetate may be responsible in part for the beneficial effect of pectin supplementation *in vivo*. Veldman *et al.* (1999) could not find a significant change in plasma fibrinogen levels with acetate supplementation, but the fibrin network architecture was less atherogenic.

Except for the limited observations mentioned above, no data could be found regarding the effect of SCFAs on haemostatic factors in human subjects. However, it is evident from the limited observations that there is a possible association between NSP intake and fibrinogen levels. This observation, however, lacks thorough investigation.

2.8 FIBRINOGEN

2.8.1 Introduction

Fibrinogen is defined as that soluble protein in blood and tissue extract, which, in the presence of thrombin, is transformed into the insoluble fibrin network structure (Blombäck, 1996). Fibrinogen is a known risk factor for CHD (Sweetnam *et al*., 1996) and is central to haemostatic events (Kloczewiak *et al*., 1984). The term ‘haemostasis’ means prevention of blood loss (Guyton, 1991, p.390). Abnormal haemostasis, and specially a pre-thrombotic state characterised by hypercoagulability, increased platelet aggregation and impaired fibrinolysis, is associated with increased atheroma and

thrombosis (Vorster *et al.*, 1997a; Sueishi *et al*., 1998). Fibrinogen is necessary for the formation of fibrin in the final step of coagulation (Junqueria *et al*., 1995, p. 219), and is also involved in the processes of fibrinolysis (Blombäck, 1996). Factors, which raise plasma fibrinogen, include smoking and tissue damage (Swales & De Bono, 1993, p.142).

2.8.2 Structure

Fibrinogen is a soluble, sulphated, acid-glycoprotein consisting of two identical halves, each containing 3 non-identical sub-units, the Aα, Bβ and YY[1] polypeptide chains, held together by disulphide bonds (Fig. 2.3). Fibrinogen has a trinodular structure with two peripheral nodules, the D-domains, connected to a central nodule, the E-domain, by triple-stranded α-helices. Fibrinogen is an elongated molecule (450Å) with a molecular weight of 340 x 10^3 and a half-life of three to four days. It is synthesised in the liver and the single copy genes of the consistent chains are located in a 46 kilobase stretch of chromosome 4. Not much is known about the regulation of synthesis and plasma levels of fibrinogen. It seems that a transcription factor (hepatic nuclear factor-1) is necessary for expression in the liver, and that the hepatocyte stimulating factors, as well as interleukin-6 produced by macrophages after stimulation by fibrin(ogen) degradation products, stimulate synthesis (reviewed by Vorster & Venter, 1994).

2.8.3 Factors influencing fibrinogen levels

Plasma fibrinogen levels reflect the balance between production (synthesis and secretion) and removal of fibrinogen from the circulation. The latter includes catabolism, conversion to fibrin, uptake by platelets and possibly other tissues, and plasmin degradation of fibrin(ogen). Plasma fibrinogen levels rise during the acute phase. It is therefore important to exclude conditions that elicit the acute-phase

Fig 2.3 **Schematic drawing of the fibrinogen molecule** (adapted from Cook & Ubben, 1990)

response when evaluating fibrinogen levels (Vorster & Venter, 1994). A number of studies support the contention that lifestyle and other factors influence plasma fibrinogen levels.

Several epidemiological studies agree that smoking has the strongest elevating effect on fibrinogen (Wilhelmsen *et al*., 1984; Stone & Thorp, 1985; Meade *et al*., 1986; Kannel *et al*, 1987; Yarnell *et al*., 1991). Fibrinogen levels are significantly higher in women than in men (Prisco *et al*., 1996), especially after the menopause or with use of oral contraceptives (Vorster & Venter, 1994). Many studies have reported that

fibrinogen levels increase with age (Armani *et al*., 1992), and westernised black men also have higher fibrinogen levels (Venter *et al*., 1992). According to El-Sayed (1996), it is suggested that short-term exercise activates coagulation and fibrinolysis and the delicate balance between clot formation and clot dissolution is maintained in normal populations. Individuals who experience job strain or stress (Ishizaki *et al*., 1996), or who are from a lower social class (Markowe *et al.*, 1985) tend to have higher fibrinogen levels, possibly because of a low social integration or lack of an extended social support network (Rosengren *et al.*, 1992). Venter *et al.* (1992) found that Westernisation of blacks is associated with an increase in fibrinogen levels. Selected groups of rural blacks had levels similar to whites and significantly lower values than urbanised blacks.

Several abnormal physiological and disease states, in their own right independent risk factors for CHD, are associated with elevated plasma fibrinogen levels. Studies in Table 2.14 highlight that hypertension, obesity, hyperlipidaemia, and diabetes mellitus are associated with elevated fibrinogen levels.

There is some evidence that diet could influence fibrinogen levels (Table 2.3). It seems that low fibrinogen levels are associated with moderate alcohol intake (Folsom, 1992). Studies on animal models fed westernised high-fat diets showed that soluble NSP may also lower fibrinogen levels (Venter *et al*., 1991). Veldman *et al*. (1997) found that the percentage of fibrinogen converted to fibrin decreased significantly after four weeks of pectin supplementation. No significant change, however, was observed in total fibrinogen levels. Vorster *et al*. (1997a) reviewed a number of studies which also show a beneficial effect of NSP on coagulation and fibrinolysis.

It is known that high levels of circulating FFA (Pickart & Thaler, 1980) and insulin resistance (Mansfield *et al*., 1996) are associated with increased plasma fibrinogen levels. It is not inconceivable that dietary interventions that reduce FFA levels and improve insulin sensitivity (such as increased NSP, increased meal frequency and decreased fat intake) could influence fibrinogen levels (Vorster & Venter, 1994).

Table 2.14 Studies indicating diseases associated with elevated fibrinogen levels

Resources	Relationship between disease states and plasma fibrinogen
Hypertension	
Wilhelmsen *et al*., 1984 Kannel *et al*., 1987 Lip *et al*., 1997	• Hypertensive patients have higher plasma fibrinogen levels.
Obesity	
Tarallo *et al*., 1992 Prisco *et al*., 1996	• Fibrinogen correlates with BMI.
Hyperlipidaemia	
Ko *et al*. 1997	• Close relationship between plasma fibrinogen and abnormal lipid metabolism.
Prisco *et al*., 1996	• Fibrinogen correlated with total cholesterol.
Møller & Kirstensen, 1991	• Fibrinogen correlated with high LDL cholesterol and low HDL cholesterol.
Simpsom *et al*., 1983	• Fibrinogen correlated with hypertriglyceridaemia.
Diabetes Mellitus	
Grau *et al*., 1996	• Diabetes mellitus independently increases fibrinogen.
Ko *et al*. 1997	• Close relationship between plasma fibrinogen and abnormal glucose metabolism.
Mansfield *et al*. 1996	• Abnormalities of circulating haemostatic factors (fibrinogen and FVIIc), possibly in relation to insulin resistance, may contribute to cardiovascular risk in relatives of patients with NIDDM.
Bruno *et al*., 1996	• NIDDM have a high prevalence of hyperfibrinogenaemia

2.8.4 Coagulation

Blood coagulation is an autocatalytic, self-limiting process that is triggered by tissue injury. It requires the sequential 'cascade' activation of a series of proteinase zymogens of which some combine with a protein cofactor on membrane surfaces for further activation, culminating in the formation of thrombin from prothrombin, with release of prothrombin fragments 1 + 2 of the fibrinogen molecule in Fig 2.3 (Vorster *et al.,* 1997). The formation of thrombin leads to the conversion of soluble plasma fibrinogen to insoluble fibrin. The process involves the release of two pairs of polypeptides from each fibrinogen molecule. The remaining portion, fibrin monomer, then polymerises with

other monomer molecules to form fibrin. This reaction is catalysed by factor XIII, the fibrin- stabilising factor, and requires Ca^{2+} (Ganong, 1993, p. 490). The fibrin forms a three-dimensional network of fibres trapping red cells, leukocytes and platelets to form a blood clot or thrombus (Junqueria *et al.*, 1995, p.233). Blood coagulation can occur either as a result of activation of the intrinsic mechanism, e.g. clotting in a tube after a clean venepuncture, or as a result of a combination of the extrinsic and intrinsic mechanisms which normally happens following injury *in vivo* (Thomson & Cotton, 1974 p.772). The many factors involved in coagulation are diagrammatically represented in (Fig 2.4).

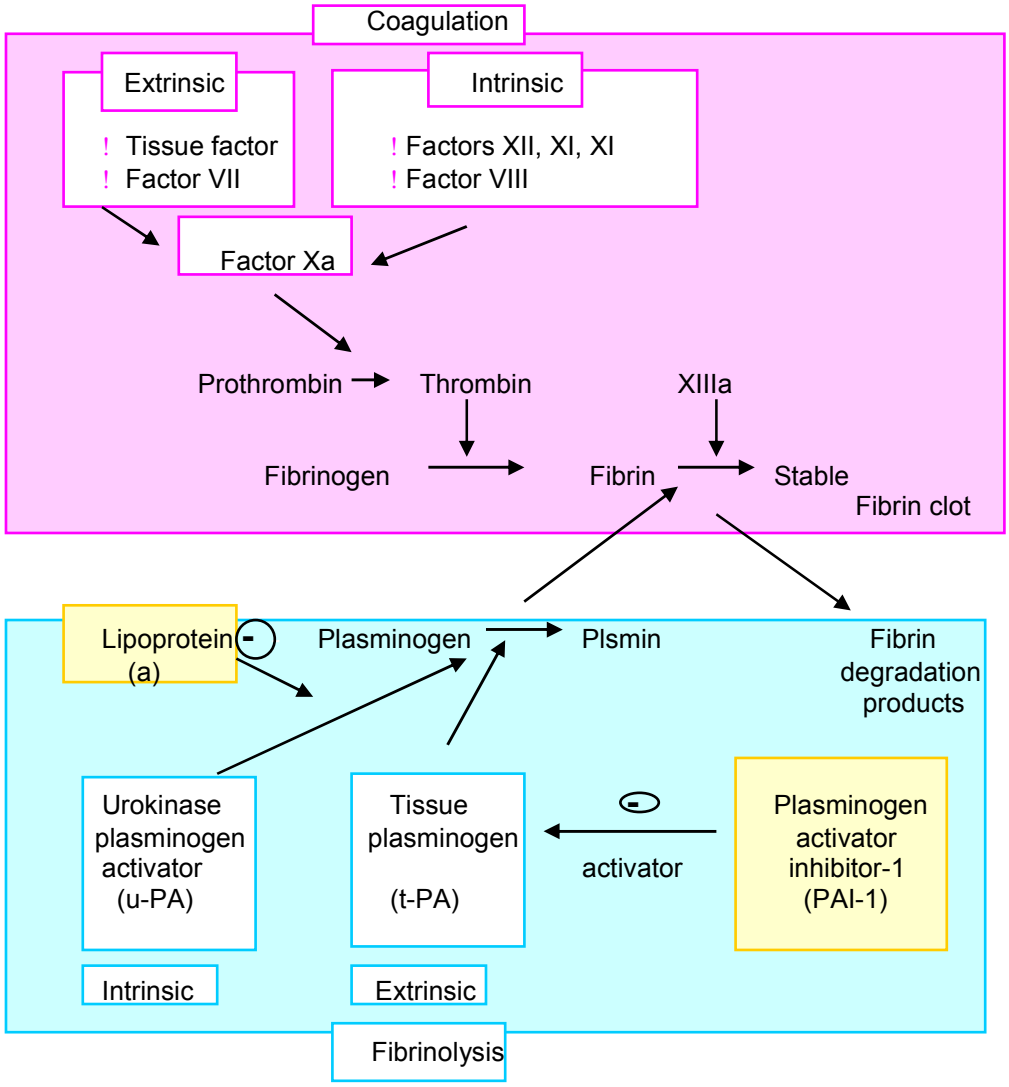


Fig 2.4 **Normal pathways of coagulation and fibrinolysis** (adapted from Pickup & Williams, 1997, p. 56.9).

Abnormal haemostasis, characterised by hypocoagulability, may lead to bleeding disorders, while hypercoagulability may result in intravascular coagulation, atherosclerosis and thrombosis (Vorster *et al*., 1988). Alessandri *et al*. (1996) showed a relationship between serum cholesterol and the rate of thrombin generation supporting the hypothesis that a hypercoagulable state may occur in hypercholesterolaemic subjects. The coagulation mechanism is normally active at a low level, even without tissue injury. Small elevations in coagulation factors activities and concentrations give rise to hypercoagulability (Miller *et al*., 1992). Hypercoagulability may be the result of an increased concentration or activity of the coagulation factors and/or decreased levels of the coagulation inhibitors and/or decreased fibrinolytic activity (Vorster *et al*., 1988). Raised fibrinogen concentrations and FVIIc are the two clotting factors which have been identified in epidemiological and clinical studies as risk factors for cardiovascular disease (Vorster & Venter, 1994; Mennen *et al*, 1996) and which may be influenced by diet (Vorster *et al*., 1997a).

2.8.5 Fibrinolysis

Plasmin is the active component of the fibrinolytic system (Ganong, 1993, p.491). Plasmin is generated from its precursor plasminogen in a restricted way because of specific interactions between fibrin(ogen), plasminogen, and various activators (Ny *et al*., 1993). Plasmin lysis fibrin and fibrinogen, with the production of fibrinogen degradation products (FDP) that inhibit thrombin (Ganong, 1993, p.491). Two types of plasminogen activators, tissue type (*t*-Pa) and urokinase type (*u*-Pa) are responsible for the conversion of inactive plasminogen to active plasmin. The fibrinolytic process (Fig. 2.4) is further regulated and influenced by the concentrations and activities of α-2-antiplasmin, plasminogen activator inhibitors (PAI-1 and PAI-2) as well as thrombin and fibrin itself (Van der Bom *et al*., 1996). It is also known that fibrinogen has the ability to inhibit plasmin function. The mechanism is mediated through cleavage between fibrinogen and plasmin at sites different from the active cleavage sites. This can only be

terminated through a rise in fibrinogen concentration and/or the formation of fibrin strands (Higazi & Mayer, 1990).

High concentrations and activities of PAI-1, possibly because of increased insulin secretion (Vague *et al*., 1986) or insulin resistance (Juhan-Vague *et al*., 1993), have been shown repeatedly in epidemiological and clinical studies to be a risk factor for atherosclerosis and thrombosis (Aznar & Estelles, 1994). Streptokinase, a bacterial enzyme, and urikinase, an enzyme produced by the kidney cells, are also fibrinolytic and are used in the treatment of early MI (Ganong, 1993, p. 491). It has also been suggested that the potent atherogenic properties of Lp (a) may be due to the structural resemblance of its Apo(a) to plasminogen, which may interfere with plasmin generation and therefore fibrinolysis (Eaton *et al*., 1987). In haemorrhagic patients, laboratory tests may demonstrate the existence of increased plasmin activity and the presence of specific congenital defects leading to primary hyperfibrinolysis, which could play a role in thrombotic disorders (Prisco *et al*., 1998).

According to Brommer *et al*. (1997), lifestyle can modify fibrinolysis. Physical training, moderate eating habits, no smoking, and moderate alcohol intake will have a beneficial effect on fibrinolysis.

2.8.6 Possible relationship between fibrinogen, thrombosis and atherogenesis

CHD is in most instances due to obstruction of coronary vessels by atherosclerosis or thrombosis, singly or in combination (Ulbricht & Southgate, 1991). The thrombogenic model of atherosclerosis (Duguid, 1946), combining the risk associated with thrombosis and atherogenesis, was one of the first models contradicting the existing lipidaemic models. Hypercoagulability is a prerequisite for the development of thrombosis and atherosclerosis (Hamsten *et al*., 1986). The haemorheologic consequences of hyperfibrinogenaemia might act at various levels: by reducing flow, by predisposing to thrombosis, and by enhancing atherogenesis (Ernst *et al*., 1986; Koenig & Ernst, 1992). This supports several other studies which also explained that fibrinogen is directly

involved in the processes of both thrombosis and atherogenesis (Naito *et al*., 1990; Sing *et al*., 1990).

The development of an atherosclerotic plaque is a time-consuming process, commencing from early lesions at childhood to advanced plaques in the adult (Ross, 1986). Atherosclerotic plaques are known to contain fibrin(ogen) (Vorster & Venter, 1994). Sueishi *et al*. (1998) support the hypothesis that the coagulation and fibrinolytic systems play an essential role in the initiation and progression of atherosclerosis through fibrin deposits both in atherosclerotic plaques and on the arterial surface by neointimal hypercoagulability and hypofibrinolysis. The possible mechanisms of fibrinogen and vascular diseases such as atherosclerosis, CHD, and stroke have been the subject of a number of hypotheses and reviews (Vorster & Venter, 1994).

The modification of fibrin may be one of the possible mechanisms whereby elevated fibrinogen translates into higher incidence of atherosclerosis (Shats *et al*., 1997). Fibrinogen converted to fibrin and FDP, when in contact with the arterial wall, could initiate and enhance the development of atherosclerosis by causing endothelial cell disorganisation and a 'breakdown' of the endothelial barrier, resulting in an increased permeability to plasma proteins and lipoproteins (Vorster & Venter, 1994). Fibrinogen and FDP have been shown to stimulate proliferation and migration of smooth muscle cells (Baller *et al*., 1995). These effects suggest that fibrinogen is involved in the earliest stages of plaque formation (Ernst & Resch, 1993).

Platelet hyperaggregation plays an accepted role in the genesis of an atherosclerotic lesion. Fibrinogen binds on the receptors on the platelet membrane, which in turn, is a precondition for aggregation *in vivo* (Cook & Ubben, 1990). Several aspects of atherosclerosis bear similarities to an inflammatory process (Ernst *et al.*, 1987). Fibrinogen as an acute-phase reactant (Ernst & Resch, 1993) enhances chemotaxis of inflammatory cells, vasoconstriction and cell proliferation (Vorster & Venter, 1994). According to Ernst and Resch (1993), it is thus conceivable that early atherosclerosis itself leads to a mild inflammatory process that elevates acute-phase proteins and other

variables of the acute-phase response. These effects could all contribute to the atherosclerotic process, while fibrinogen's role in blood viscosity, platelet aggregation and blood coagulation will contribute to thrombus formation and occlusion of blood vessels (Vorster & Venter, 1994).

2.9 SUMMARY

It is evident that raised fibrinogen concentration, the fibrin network architecture, and other haemostatic risk factors should be regarded as serious risk factors for atherosclerotic and thrombotic vascular disease. Many studies have indicated that both reversible and irreversible risk factors are associated with heamostatic risk factors. A change in lifestyle may decrease the risk for CHD.

Diet is one of the controllable factors in the treatment and prevention of degenerative western diseases, such as CHD. On the basis of current understanding, it is apparent that western diets do not contain enough NSP and that the average intake falls well below the recommended level. From several studies it is evident that water-soluble fibre may increase glucose tolerance, reduce serum lipid and lipoprotein concentrations, fibrinogen concentrations, and change the fibrin network architecture.

Fermentation of NSP results in the production of SCFAs, which probably have beneficial effects on haemostatic and other coronary risk factors.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

The main objective of this study was to examine the effect of a combination of SCFAs on plasma fibrinogen concentrations and related risk factors in westernised black men of the SANDF. To reach this goal, the study design, selection of subjects, measurements, intervention, statistical analysis as well as the limitations of the study will be discussed in this chapter. The study was approved by the Ethics Committee of the UOFS (Ethics number: 58/98). All subjects participating in the study gave their written consent (Appendix 1).

3.2 STUDY DESIGN

The study design (Fig 3.1) was that of a randomised, placebo-controlled, double-blind, clinical trial. A clinical trial is a well-controlled study designed to address a question of relevance to human health and the investigator has control over the assignment of subjects to one or more treatment and comparison or control groups (Dennis & Kris-Etherton, 199, p.151). Subjects falling within a pre-determined set of inclusion criteria were included in the study. According to these criteria, subjects were divided by a randomisation list into two groups, the experimental group and the control (placebo) group with 11 subjects in each group. The subjects received either a placebo or a SCFA supplement in the form of a capsule, coated with a thin layer of chelac. Supplementation was sustained for 5 weeks. Measurements in the form of questionnaires, blood pressure, anthropometric and biochemical analyses were taken at baseline (week 0) and at the end of the study (week 5). The study was undertaken at a time specifically designed to minimise seasonal effect on human metabolism.

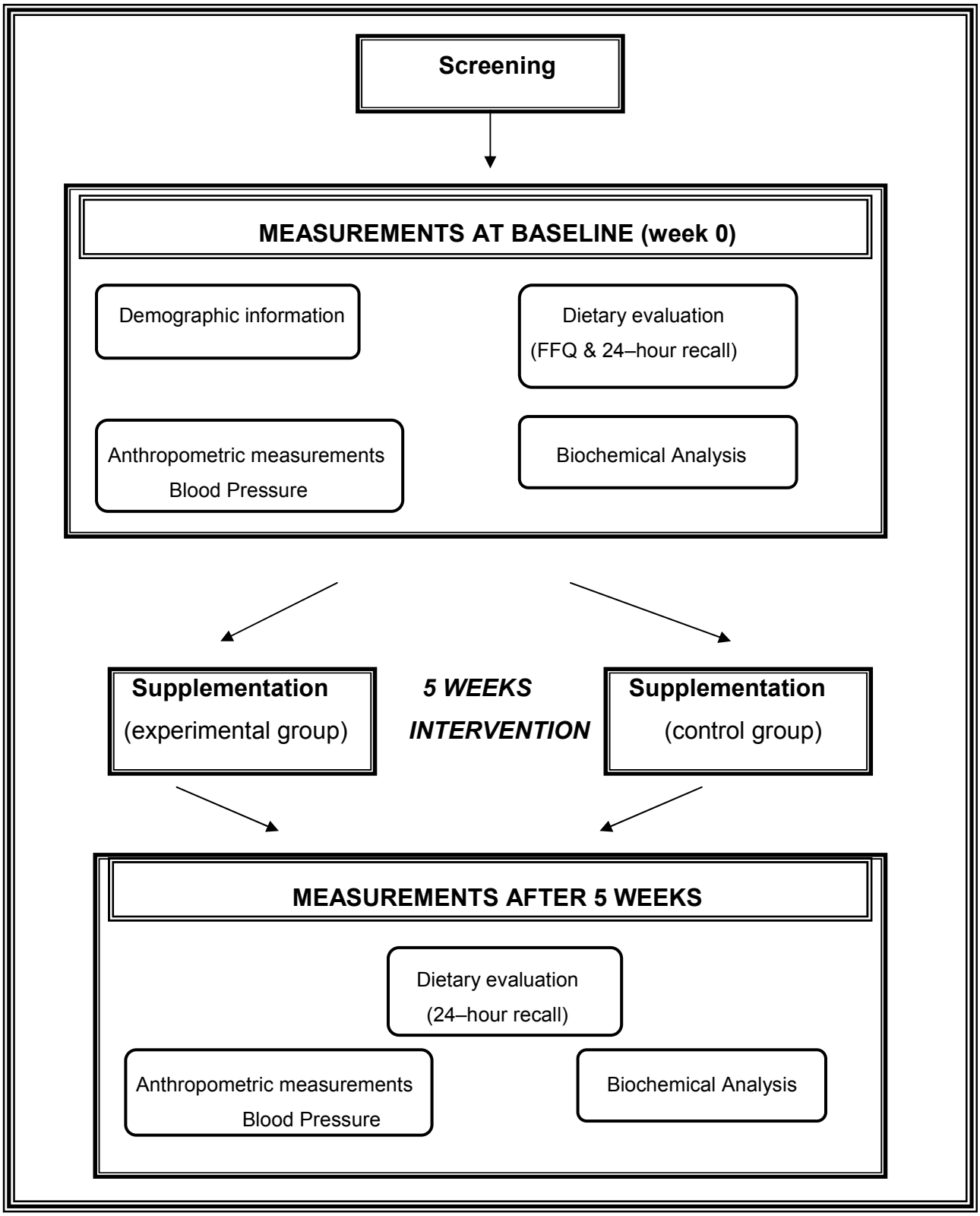


Fig 3.1 Experimental design of the study

3.3 SAMPLE

Permanent Black members inhabiting the same quarters of the SANDF at Tempe, Bloemfontein, was recruited for this study. Volunteers were recruited according to inclusion criteria.

Because of the intensive nature of the present study ten to fifteen subjects in both the experimental and placebo groups were at first decided on. Although ten to fifteen subjects per group were a relative small sample size, it was considered large enough to have sufficient statistical power to reach the aims of the study. Furthermore network studies where fresh plasma is used requires intensive attention and hands to do the analysis which make it impractical to use large sample sizes (20 to 30 per group). In various other studies on fibrin network structures small numbers were used, e.g. 5 to 6 subjects (Nair *et al*., 1991; Blombäck *et al*, 1992) and 10 subjects per experimental and placebo group used by Veldman *et al*. (1999).

3.3.1 Inclusion criteria

According to Dennis & Kris-Etherton (1991, p.153), results from human studies are frequently difficult to interpret because of the heterogeneity in human populations and the variability in free living conditions. A concerted effort must be made to control these factors in the design of a clinical trial, beginning with the subject selection criteria. Therefore, the choice of subjects included into the study was based on the following inclusion criteria:

- Black males aged between 20 and 45 years.
- No history of any previous cardiovascular incidences should be evident.
- Subjects should not use any chronic medication, including hypercholesterolaemic drugs, or have diseases of the liver, kidneys, heart, etc.
- The background diet should be similar – all the subjects were eating all their meals at the same dining room (mess).

- No smokers.
- No alcohol-dependent subjects.
- Subjects should not take any medication or supplementation during the study without the knowledge of the researcher.
- Subjects should not undergo any changes in lifestyle during the study, avoid or minimise any agents that may have had an influence on their cardiovascular risk profile, such as changing dietary habits, exercise, using alcohol, aspirin, etc.
- Subjects would have to be permanent staff of the SANDF for at least one year prior to the study.
- Fibrinogen levels that could decrease (> 2.5 g/L).

Eleven subjects were randomly allocated to each, the experimental group or control group.

3.3.2 Screening

Screening ensures that participants meet eligibility criteria and are able to comply with the requirements of the study (Dennis & Kris-Etherton, 1991, p. 155). Screening for this study took place during an individual interview, using a standardised questionnaire (Appendix 2) to gather the information needed according to the inclusion criteria. A trained fieldworker was available during the interview to translate any issues that the subjects might not have understood. Blood samples were also taken to screen subjects for fibrinogen levels.

In compliance with the inclusion criteria only 22 volunteers, who were all eating at the same mess of the School of Armour, were recruited to voluntarily participate in the study. Each participant in the study received written information about the study (Appendix 3).

3.4 MEASUREMENTS

The first and very important step in measurement is to define objectives – determine exactly *what* is to be measured. Another part of the measurement process is to decide *how* to do the measuring – to select or develop an instrument that will best perform the task (Compton & Hall, 1972, p. 199). Suitable variables for this study according to the literature were selected to answer to the question as to *what* is to be measured and standardised techniques were selected to answer to the question as to *how* to do the measurement.

3.4.1 VARIABLES

Variables defined for the aim of this study were metabolic indicators, coronary risk factors, haemostatic risk factors, acetate and dietary indicators.

3.4.1.1 Metabolic indicators

The glucose tolerance and other metabolic indicators were used to indicate general health of the subject group and were measured at baseline and at the end of the study.

i. **Fasting glucose** refers to the glucose levels where fasting blood glucose levels of < 6.0 mmol/l were taken as normal glucose levels. Considering that the subjects had normal glucose according to the inclusion criteria, no effect on carbohydrate metabolism was expected (South African Institute for Medical Research (SAIMR), 1991).

ii. **Other metabolic variables** refer in this study to total protein, albumin, haematocrit, haemoglobin, white and red blood cell count which in the literature are used as markers for health and nutritional status (Lindsey, 1996, p. 179, 507). Normal ranges for black populations, according to the Chemical Pathology and Haematology Department at the UOFS, are presented in Table 3.1.

3.4.1.2 Coronary risk factors

In this study coronary risk factors were divided into irreversible and reversible risk factors.

Table 3.1 Normal ranges for metabolic indicators used in this study

Metabolic indicator	Normal range
Total protein:	64 – 84 g/L[a]
Albumin:	34 – 52g/L[a]
Haematocrit	41 –52 %[b]
Haemoglobin	13.7 – 17.8 g/dL[b]
White blood cell count	2.53 – 8.43 x10 ^{9}/L[b]
Red blood cell count	4.49 – 5.90 x10 ^{12}/L[b]

[a] Chemical Pathology UOFS, 1999

[b] Haematology Department UOFS, 1997

i) Irreversible risk factors

The irreversible risk factors represented the age, sex, the presence of a history of previous CHD of the subject and a family history of hypertension, hypercholesterolaemia, previous CHD, diabetes mellitus and stroke.

ii) Reversible risk factors

Anthropometric status, hypertension and dyslipidaemia represented the reversible risk factors.

a. **Anthropometry** in this study refers to body mass index (BMI) and waist-to-hip ratio (WHR).

1. **BMI** refers to a relationship of weight in kilogram to the square of the height in meter (Weight (kg)/ height^2 (m)^2) as described by Jooste *et al*. (1988) and Steyn *et al*. (1990).

Underweight	< 20 kg/m^2
Normal weight	21 $\geq\leq$ 25 kg/m^2
Overweight	>25 kg/m^2
Obesity	$\geq$ 30 kg/m^2

2. **WHR** refers to $\frac{\text{waist circumference (cm)}}{\text{hip circumference (cm)}}$.

According to Charlton *et al*. (1996) indicates a WHR > 1.0 android obesity for men.

b. **Hypertension** as indicated by Rossouw *et al*. (1983), Steyn *et al*. (1991) and Seedat *et al*. (1993) refers to high arterial blood pressure, represented by raised systolic and diastolic blood pressure.

Normal blood pressure	<140/90 mm Hg
Borderline hypertension	140/90 mm Hg >< 160/95 Hg
Hypertension	>160/95 mm Hg

c. **Dyslipidaemia** refers to raised serum cholesterol, LDL cholesterol, serum triglycerides and low HDL cholesterol.

Table 3.2 Action limits for dyslipidaemia (Institute for Pathology, University of Pretoria, 1999).

	Total s-cholesterol	LDL-cholesterol	S-triglycerides	HDL cholesterol
Normal:	3.0 - 5.2 mmol/l	2.0 - 3.4 mmol/l	0.3 - 2.0 mmol/l	0.9 - 1.6 mmol/l
Raised risk:	>5.2 mmol/l	>3.4 mmol/l	>2.0 mmol/l	< 1.6 mmol/l

3.4.1.3 Haemostatic risk factors

Haemostatic risk factors are represented in this study by fibrinogen, fibrin network architecture, factor VII, factor VIII, CRP and fibrin monomers. The fibrin network content (FNC), compaction of fibrin networks as well as the mass to length ratio of fibrin fibres (μ_T) and network lysis rate were measured to determine the characteristics of the fibrin network architecture. Where applicable, the normal ranges for the haematologic variables as provided by the manufacturer of the specific standardised techniques used are presented in Table 3.3.

Table 3.3 Ranges for haemostatic risk factors as provided by the supplier of the techniques used.

Fibrinogen	1.8 – 3.5 g/L	
Fibrin monomers	3.4 –14.5 mg/L	
Factor VII & Factor VII	Between 70-150% of normal factor VII and VIII activity (100%).	
CRP	< 0.01g/L	Exclude many acute inflammatory diseases, but do not especially exclude inflammatory processes.
	< 0.05 g/L	Occur in acute diseases in the presence of slight to moderate inflammatory processes.
	> 0.05g/L	High and extensive inflammatory activity.
Fibrin network content, compactions, mass to length ratio, lysis rate	No normal ranges available	

3.4.1.4 Acetate

Acetate concentration refers to fasting acetate concentration at baseline and after five weeks of SCFA supplementation.

Normal fasting acetate: < 50μmol/L (Cummings *et al.,* 1987a).

3.4.1.5 Dietary indicators

i. Energy, macro- and micronutrient intake

For the aim of this study, dietary indicators were mainly represented by the energy, macro- and micronutrient intake of the subject. The prudent diet guidelines were used as reference for macronutrient intake (Wolmarans *et al.*, 1988), and RDAs (recommended dietary allowances) were used as reference for energy and micronutrient intake. The RDA as a component of the DRIs was used in the present study and is the daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97 – 98 %) individuals in a given life-stage and gender group (VIC, 1999). The term dietary reference intakes (RDIs) is a collective name and refers to a set of at least four nutrient-based reference values where each of these reference values has special use (VIC, 1999):

- EAR: Estimated Average Requirement
- RDA: Recommended Dietary Allowance
- AI: Adequate Intake
- UL: Tolerable Upper Intake Level

The references used to indicate an atherogenic diet for this study according to the prudent guidelines and RDA include:

- Total daily energy intake indicated in kilojoules determined according to activity level;
- total amount of energy represented by fat in the diet of more than thirty percent;
  - total amount of energy represented by SFA in the diet of more than eight percent;
  - total amount of energy represented by MUFA in the diet of less than twelve percent;

    - total amount of energy represented by PUFA in the diet of less than ten percent;
  - total amount of energy represented by protein in the diet of more than 0.8g/kg ideal body weight;
  - total amount of energy represented by carbohydrates in the diet of less than fifty percent;
    - total amount of energy represented by added sugar to the diet of more than ten percent;
- total daily intake of dietary fibre of less than 20 g.
- micronutrients less than 67 percent of the RDA.

ii. **Alcohol intake** refers to the use or non-use of alcohol by the subject as well as the type of drink, the frequency and the quantity used. Information about alcohol consumption was obtained as described by Grønbæk & Heitmann (1996) for the frequency and the quantity of the average number of drinks of beer (cans), wine (glasses) and spirits (units). Subjects were divided into four groups according to their alcohol intake:

a) Abstainers (< 1 drink / week)
b) Light drinkers (1-6 drinks per week)
c) Moderate drinkers (7 – 21 drinks per week)
d) Heavy drinkers (> 21 drinks per week).

It is important to note that heavy drinkers were not included in the study.

3.4.2 Techniques

Validity describes the degree to which a dietary method measures what it purports to measure (Gibson, 1990) and *reliability* is defined as the degree to which a method yields the same results on two different occasions or by two different people (Lee-Han *et al*., 1989). Asking the question “Are we measuring what we think we are measuring?”

ensured validity of the measurements (Compton & Hall, 1972, p. 201). To collect all the necessary information, specific variables were measured according to specific techniques. The techniques were standardised and precautions were taken to ensure the collection of valid and reliable information. The techniques used in this study were questionnaires, anthropometric measurements, blood pressure and biochemical analyses.

3.4.2.1 Questionnaires

Questionnaires used in this study included:

i) screening (Appendix 2);
ii) demographic (Appendix 4);
iii) food frequency questionnaire (Appendix 5);
iv) 24-hour recall (Appendix 6);
v) tolerance questionnaire (Appendix 7).

i) Demographic questionnaire

During an individual interview, background information on the irreversible and some of the reversible risk factors used in this study was obtained with the aid of a questionnaire. The questionnaire included questions about the age, activity level, smoking habits, alcohol intake, medical history of the subjects and prevalence of CHD or coronary risk factors in family members of the subjects.

ii) Dietary Questionnaire

The aim of the dietary questionnaires used in this study was to measure the usual dietary intake of the subjects as well as to determine whether any dietary changes occurred after the intervention.

a. Usual dietary intake

The FFQ and 24-hour recall were used to obtain the usual dietary intake of the subjects. Over-reporting for the FFQ and under-reporting for the 24-hour recall is described by the literature as shortcomings for these questionnaires (Katzenllenbogen *et al*., 1999). Therefore, both the FFQ and 24-hour recall were used and compared to increase the relative validity of the information obtained.

Food Frequency Questionnaire

According to the literature, the diet history and food frequency questionnaire (FFQ) are the most accurate methods of evaluating usual intake (Gibson, 1990; Dwyer, 1994, p.847; Goldbohm *et al*., 1995). Bingham *et al*. (1994) indicated that a well-designed and administered FFQ would have the same results as a diet history. The FFQ was designed for this study to measure the usual dietary intake of this specific study group as follows:

- An already validated questionnaire used for black populations in the Free State was used and compared and extended according to the menu's, ratio scale and standard portion sizes used in the mess.
- A 24-hour recall was also done on some members eating at the mess to incorporate snacks and other food items not listed in the FFQ.
- The pre-coded FFQ included a list of foods which were grouped according to similarities in type (e.g., cereals, fruits, meats, dairy products, etc.).
- Additional information required to code the questionnaire included questions asked about food preparation, cooking methods and the addition of foods as well as combination dishes.

The subject was required to estimate the usual frequency of consumption of a range of foods in terms of times per month, week or day. Questions on frequency and type of fat added in cooking or at the table, the skin of the chicken and the fat of the meat were

asked. Questions on frequency of fruit and vegetables intake and when possible whether fruits were eaten with the skin were also asked. Open-ended questions, asking about foods eaten more than once a month and not listed in the FFQ, were also included.

The FFQ was designed according to the literature (Block *et al*., 1986; Lee-Han *et al*., 1989; Wheeler *et al*., 1994; Young & Nestle, 1995; Kumanyika *et al*., 1996; Wolk *et al*.,1998). The validated well-designed FFQ was considered suitable to determine the usual intake of the subjects in this study.

24-Hour Recall

The 24-hour recall can be used to form a general picture of the usual food intake, but a single day's intake is usually inadequate to characterise an individual's usual intake (Dwyer, 1994, p.852). A 24-hour recall is considered suitable on samples of 50 or more respondents, but if a homogeneous study group is used, a single 24-hour recall can be done on fewer individuals to evaluate usual dietary intake.

The 24-hour recall indicated the actual food consumption during the previous 24-hour period. The 24-hour recall was an open-ended questionnaire which included questions about the time food was eaten, the preparation and the portion size, when possible in ordinary units as used in the mess. A question was also included where the subject was asked if he normally ate as on the specific day the 24-hour recall was taken.

b. Dietary change

The same 24-hour recall used at baseline was used at the end of the study to determine if any change occurred in the usual dietary intake of the subjects.

c. Limitations of the questionnaires and precautions taken to overcome the limitations.

Important errors in dietary surveys are that people do not remember what items of food they eat, and they do not accurately estimate portion size (Garrow, 1995). To answer the questions of validity and reliability, the performance of dietary questionnaires depends on the answers to two broad questions: 1) how accurately can the individual report on his frequency of consumption and his portion sizes? And 2) how adequate is the food list itself, and its associated quantification (Block *et al*., 1986). Table 3.4 indicates the limitations of the FFQ and 24-hour recall as summarised by Dwyer (1994, p.847) as well as the precautions taken in the present study to overcome the limitations and increase the validity and reliability of the questionnaire.

Considering all the mentioned measures to overcome the limitations of the FFQ and 24-hour recall, the chosen dietary questionnaires can be taken as a reliable method and validation was done during the pilot study.

iii) Tolerance questionnaire

The tolerance questionnaire was completed after the study to determine whether any of the subjects experienced any side-effects from the intervention. Questions were asked on nausea, constipation, diarrhoea, decrease or increase in appetite, and whether the amount of capsules consumed was acceptable.

3.4.2.2 Anthropometric measurements

BMI and WHR were used to determine the body composition of the subjects. The anthropometric measurements included weight, height, waist and hip measurements. Weight and height were determined to calculate the BMI and waist and hip were determined to calculate the WHR.

Table 3.4 Limitations of the dietary questionnaires and precautions taken to overcome the limitations

Limitations of the FFQ	Precautions to overcome limitations
FFQ	
▪ Incomplete response may be given.	▪ All the subjects were living-in members and their response was monitored by the menu-book used in the mess. ▪ The portion sizes used in the FFQ were confirmed with the portion sizes used in the mess according to the ratio scale of the SANDF.
▪ Lists compiled for the general population are not useful for obtaining information on groups with different eating patterns.	▪ The questionnaire was designed considering the cultural eating patterns of the subjects as well as the usual menu used in the mess.
▪ Respondent burden rises as the number of food items queried increases.	▪ The subjects were informed on how long the questionnaire would take to fill in. The questionnaire was obtained in the form of an interview. This decreased the burden on the subjects.
▪ Foods differ in extent to which they are over- and under-reported.	▪ Three dimensional food models, portion sizes, cups, plates and spoons usually used in the mess were used to estimate and confirm the portion sizes. ▪ Open-ended questions were asked for any foods eaten at least once a week that did not appear in the questionnaire.
▪ Translation of food groups to nutrient intakes requires that many assumptions be made.	▪ The composition of combined meals as well as additions usually made to specific foods were determined beforehand and incorporated in the questionnaire.
24-hour recall	
▪ Inability to recall and estimate the portion size of the food consumed	▪ The menu of the previous day was obtained from the mess to control the response of the subject.
▪ Selective forgetting of foods such as liquids, high-calorie snacks, alcohol and fat occurs.	▪ Specific questions on additional food items not available in the mess were asked.
▪ It does not reflect differences in intake for weekday vs. weekend.	▪ The subject was asked if he normally eats as on that specific day.

i) Weight

Body weight was measured according to a standard method described by Jensen *et al*. (1983, p. 45) and Charlton *et al*. (1996) with a Seca digital electronic scale to the nearest 0.1kg. The weight of the subjects, wearing light clothing and no shoes, was measured before blood samples were collected before breakfast and after the subjects went to the toilet. The weight was measured at the same time of day at baseline and after supplementation for five weeks.

ii) Height

Standing height of subjects, wearing no shoes and light clothing, was measured to the nearest 0.5cm using a statiometer as done by Charlton *et al*. (1996). The subjects stood with their feet together, heels against the measuring board. They stood erect, neither slumped nor stretching, looking straight ahead, without tipping the head up or down. The top of the ear and outer corner of the eye were in a line parallel to the floor (the “Frankfort plane”). The top of the statiometer was lowered to rest flat on the top of the head.

iii) Waist and Hip circumferences

Waist and hip circumferences were measured in duplicate, around the smallest and widest part of the waist and hips respectively to the nearest 0,1cm. Waist circumference was measured at the smallest level of the umbilicus. Hip circumference was measured at the largest diameter below the umbilicus or maximum circumference over the buttocks, taken perpendicularly on the axial line of the trunk as described by Charlton *et al*. (1996).

3.4.2.3 Hypertension

Blood pressure was recorded with a mercurial Baumanometer before blood samples were collected, according to the methods used by Staats *et al*. (1996). The subjects were seated with the back supported, the cuff was applied to the right upper arm, and after a five-minute rest period, three intermittent readings were taken at two-minute intervals and the lowest value was recorded. Systolic blood pressure was taken at the first appearance of clear, repetitive tapping sounds (Korotkoff Phase I). Diastolic blood pressure was taken at the point of muffling of Korotkoff sounds (phase IV).

3.4.2.4 Biochemical Analyses

Biochemical information was obtained through citrate plasma (two parts citrate to nine parts blood) for fibrinogen, factor VII, factor VIII and fibrin network structures. Whole blood was clotted for the preparation of serum to determine total cholesterol, HDL cholesterol, LDL cholesterol, serum triglycerides, fasting blood glucose, CRP, albumin and total protein. The Fibrinogen Unit at the Technicon Free State (Department Paramedical Science) performed the analysis according to standardised procedures.

The coefficient of variation (CV) of the methods were determined for each set of measurements for control of accuracy and reproducibility of the methods. The CV for each method was calculated as:

CV = [Standard deviation]/[Mean] x 100

i) Sample preparation

The subject's blood samples were taken with minimum stasis using the bulldog method into evacuated glass tubes.

a. Plasma

For the preparation of essentially platelet-free plasma, 10 ml citrated blood (1 volume of 3.8 % tri-sodium citrate [Saarchem, South Africa, cat. no. 582 25 00] in a 0.1 M sodium phosphate buffer, pH 7.4, with 35 µL of 10.000 KIU/mL Trasylol®/aprotinin per 9 volumes of whole blood) was centrifuged twice for 10 minutes at 2 800 xg (Beckman Model TJ-6 Centrifuge, Beckman Instruments, Palo Alto, California, USA). Citrate acts as an inhibitor of early activation of factor V and VII. Aprotinin (Trasylol®, Bayer-Miles, Germany, Cat. no. H2912) acts as an inhibitor of fibrino(geno)lysis.

Determinations involving fibrin network structure properties and fibrinogen determinations were performed on fresh plasma samples. All remaining plasma was stored at -72 °C in Eppendorf® vials.

b. Serum

5 mL of whole blood was left to clot at room temperature. These samples were centrifuged at 2800 xg for 20 minutes in order for the serum to separate. Samples were frozen at -72 °C in Eppendorf® vials.

c. EDTA blood

5 mL blood was obtained in pre-treated K_3EDTA-tubes (VAC-U-TEST®). After using some of the samples for the determination of full blood counts the samples were centrifuged for 15 minutes at 2800 xg. The remaining samples were stored at -72°C in Eppendorf® vials.

ii) Measurement of biochemical variables

The methods used to measure the above-mentioned biochemical variables will be discussed in the next section. Standardised techniques were used to perform the measurements.

a. Total plasma fibrinogen

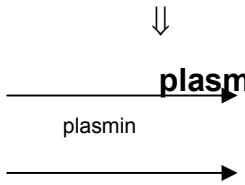
The method of Clauss (1957) was used for the quantitative determination of total plasma fibrinogen concentration.

The Clauss method : Total fibrinogen concentration was determined with the method of Clauss and a Fibrintimer^2 (Pathteq, Marburg, Germany) using Multifibren® U (14 x 2 ml code no.: OWZG 15). Kaolin Suspension (code no. OQAB) was used for the Fibrintimer^2. Citrated plasma was brought to coagulate with a large excess of thrombin. The coagulation time depends on the fibrinogen concentration in the sample. Control Plasma N (code no. ORKE) and Control Plasma P (code no. OUPZ) were used as standards for internal quality control. The C.V. for the method was 1.8%.

b. Fibrin monomers

The concentration of soluble fibrin (fibrin monomers) in plasma in order to diagnose hypercoagulable states was determined using a Berichrom® FM (code. no. OWXZ, Behring, Marburg, Germany) test kit. Soluble fibrin in the sample stimulates activation of plasminogen to plasmin by tissue plasminogen activator (tPA). The resulting plasmin was measured photometrically at 405nm via its reaction with a chromogenic substrate. To make the test insensitive to the interference by the plasmin inhibitor α_2-antiplasmin in the sample, the test utilises an elastase degradation product of plasminogen, miniplasminogen, as miniplasmin was only very slowly inhibited by the inhibitor. The C.V of the method was 6.8%.

Soluble fibrin



c. Fibrin network architecture

The compaction of fibrin networks, the mass to length ratio from turbidity, the network fibrin content and the network lysis rate were measured in order to determine the architecture of the fibrin networks of the subject group.

1. Compaction of fibrin networks

Compaction was measured in triplicate using the method as described by Dhall *et al.* (1976). The compaction technique describes the tensile strength of fibrin fibres. The inverse correlation between compaction and Young's modulus, and between compaction and strength at break, indicates that the simple method of compaction depends on the number and strength of the primary crosslinks and branch points in the network (Nair *et al.,* 1991). 0.9 mL fresh plasma was pipetted into 1.5 mL Eppendorf® vials, pre-sprayed with lecithin-based aerosol (Spray-a-Cook)® to render the surface non-adhering. The plasma was clotted by introduction of 100 µL Thrombin Reagent (ICN Biomedicals Inc., Aurora Ohio, USA, cat. no. 9002-04-4; 1 IU/mL Thrombin final concentration, 25 mM Ca^{++} final concentration). Samples were left overnight for maximum polymerisation. After centrifugation at 8000 g for 45 seconds the volume of expelled sample from the fibrin networks was determined and expressed as a percentage of the initial volume. A freshly prepared pooled plasma sample was used as control standard. The C.V. of the method was 1.5%.

2. Mass length ratio from turbidity (μ_T)

Mass length ratio from turbidity (μ_T) was determined (n=5 for each sample) using the method as described by Nair *et al*. (1991). 0.9 mL of fresh platelet-free plasma was pipetted into micro-cuvettes of 1 cm path length. The plasma was mixed and clotted by introduction of 100 µL Thrombin Reagent (ICN Biomedicals Inc., Aurora Ohio, USA, cat. no. 9002-04-4; 1 IU/mL Thrombin final concentration, 25 mM Ca^{++} final concentration).

The intercept, A, in plots of c/T(wavelength)^3 as a function of 1/(wavelength)^2 was used to calculate μ_T (mass length ratio) according to the equation:

$$\mu_T = [10/1.48xA] \times 10^{12} \text{ daltons} \qquad (\mathbf{1})$$

Turbidity (optical density) was measured at a range of wavelengths between 600 and 800 nm. The C.V. of the method was 2.9%.

3. Network fibrin content

The method of Ratnoff & Menzies (1951) was used for duplicate determination of the fibrin content of fibrin networks for all fibrin network structure determinations.

The Ratnoff & Menzies method: 0.9 mL plasma for network fibrin content in test tubes filled with 1g glass beads (Saarchem, South Africa, cat. no. 267 02 50) was clotted under identical conditions as described for mass length ratio determinations. Samples were left overnight for total polymerisation to take place. All samples were centrifuged at 1300 x g. The isolated networks were washed three times with saline solution. 1 mL 2.5 M NaOH (Saarchem, South Africa, cat. no. 582 31 80) were dispensed into each test tube, the networks dissolved by heating at 95°C for 15 minutes. The samples were left to cool at room temperature. 7 mL water and 3 mL

1.9 M sodium carbonate (Saarchem, South Africa, cat. no. 582 20 40) were dispensed into each tube, containing 200 µL of the NaOH-fibrin suspension. The tubes were vortexed and mixed with 1.0 mL Folin & Ciocalteu's Reagent (Saarchem, South Africa, cat. no. 243 300). Samples were incubated at room temperature for 20 minutes and absorbances measured at 650 nm. Different concentrations of DL-Tyrosine (Sigma, USA, St. Louis, cat. no. T-3379) dissolved in 0.1 M HCl were used to prepare a standard curve. This curve was used to calculate the concentration of fibrin present in unknown samples. The C.V. of the method was 5.6%.

4. Network lysis rate

The network lysis rate was measured in duplicate using a method developed in the laboratory of the Fibrinogen Unit of Technicon Free State. 90 µL of plasma was mixed and clotted by the introduction of 10 µL Thrombin reagent (ICN, USA, cat. no. 101141; 1IU/ml Thrombin final concentration, 25mM calcium final concentration) in microtiter plates. After total polymerisation took place, 50 µL of Streptokinase (ICN, USA, cat. no. 101141) with a final concentration of 100 U/mL was introduced to start lysis of networks. The lysis rate was measured with an EL 312e from Biotek instruments at 608 nm for 4 hours using 10 minute intervals. The lysis rate was determined by plotting the time versus change in absorbance.

d. Factor VII

The activity of coagulant factor VII was determined by coagulometric methods with a Fibrintimer^2 (Pathteq, Marburg, Germany). Plasma deficient in any one of the factors comprising the extrinsic pathway resulted in a prolonged thromboplastin time. Factor deficient was used to confirm a factor deficiency, in general, and in the identification and quantification of the factor deficient in the patient plasma. A mixture of the respective factor deficient plasma (Coagulation Factor VII Deficient plasma (human) Code no. OTXV, Dade Behring, Marburg, Germany) and the patient plasma was tested in the thromboplastin assay (Thromborel S, code no. OUHP, Dade Behring,

Marburg, Germany). The result was interpreted using a reference curve obtained with dilutions of Standard Human Plasma (code no. ORKL, Dade Behring, Marburg, Germany). Patient plasma deficient in a specific factor is not able to accommodate the absence of the factor in the corresponding factor deficient plasma and therefore produces a prolonged thromboplastin assay time. Control Plasma N (code no. ORKE, Dade Behring, Marburg, Germany) and Control Plasma P (code no. OUPZ, Dade Behring, Marburg, Germany) were used as standards. The C.V. of the method was 1.43%.

e. Factor VIII

The activity of coagulant factor VIII was determined by coagulometric methods with a Fibrintimer^2 (Pathteq, Marburg, Germany). Plasma deficient in any one of the factors comprising the intrinsic pathway resulted in a prolonged partial thromboplastin time. Factor deficient was used to confirm a factor deficiency, in general, and in the identification and quantification of the factor deficient in the patient plasma. A mixture of the respective factor deficient plasma (Coagulation Factor VIII Deficient plasma-human, code no. OTXW, Dade Behring, Marburg, Germany) and the patient plasma was tested in the thromboplastin assay (Pathromtin SL, code no. OQGS, Dade Behring, Marburg, Germany). The result was interpreted using a reference curve obtained with dilutions of Standard Human Plasma (code no. ORKL, Dade Behring, Marburg, Germany). Patient plasma deficient in a specific factor will not be able to accommodate the absence of the factor in the corresponding factor deficient plasma and therefore produce a prolonged thromboplastin assay time. Control Plasma N (code no. ORKE, Dade Behring, Marburg, Germany) and Control Plasma P (code no. OUPZ, Dade Behring, Marburg, Germany) were used as standards. The C.V. for the method was 2.09%.

f. C Reactive Protein

CRP was determined with the Boehringer Mannheim Hitachi 902 chemistry analyser (Japan) using CRP (Tina-quant® CRP,cat. no. 1551922, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany). The serum sample was mixed with a tris(hydroxymethyl)-aminomethane hydrochloride buffer and CRP antibody buffer, which started the reaction. Anti-CRP antibodies react with antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically. Addition of PEG allows the reaction to progress rapidly to the end point, increases sensitivity, and reduces the risk of samples containing excess antigen producing false negative results.

The method was calibrated against the CRP calibrator for automated systems (cat. no. 1355279, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany).

g. Serum lipids

1. Total cholesterol

Serum total cholesterol (TC) was determined with the Boehringer Mannheim Hitachi 902 chemistry analyser using an enzymatic colourimetric (CHOD PAP) method (cat. no. 1489 232, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany). The serum sample was mixed with a reagent containing enzymes, amino-antipirine, hydroxybenzoate-buffer and a surfactant-activator. The enzyme, cholesterolesterase, hydrolyses the cholesterolesters in the sample to free cholesterol. The free cholesterol produces hydrogen peroxide in the presence of oxygen and cholesterol-oxidase. The hydrogen peroxide reacts with a colouring agent, producing quianeimine, which is measured photometrically at 500 nm. The absorbance of the dye is directly equal to the cholesterol content of the sample. The concentration is expressed in mmol/L. The method was calibrated against the Calibrator for Automated Systems (c.f.a.s.) (cat. no. 759 350, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany). Precinorm U (normal values) (cat. no. 171 735, Boehringer Mannheim-Roche Diagnostics,

Mannheim, Germany) and Precipath U (abnormal values) (cat. no. 171 760, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany) were used as control serum. The C.V. for the method was 0.8%.

2. Triglycerides

Triglycerides (TG) were measured by means of the Boehringer Mannheim Hitachi 902 chemistry analyser using an enzymatic colourimetric method (GPA-PAP) (cat. no. 1488 872, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany). This method was based on the principle of formation of glycerol and free fatty acids using lipoprotein lipase (LPL). The glycerol was reduced to glycerol-3-phosphate and reacts with oxygen in the presence of glycerol-3-phosphate oxidase. Hydrogen peroxide was produced during the latter reaction. A coloured complex was formed with amino-antipirine and chlorophenol in the presence of peroxidase. The intensity of the complex was directly equal to the TG concentration in the sample. The absorbance of the sample was measured photometrically at 500 nm. Values were expressed in mmol/L. The method was calibrated against the c.f.a.s. (cat. no. 759 350, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany). Precinorm U (normal values) (cat. no. 171 735, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany) and Precipath U (abnormal values) (cat. no. 171 760, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany) were used as control serum. The C.V. for the method was 0.7%

3. HDL cholesterol

Low-density lipoproteins (LDL and VLDL) were precipitated quantitavely by the addition of phosphotungstic acid in the presence of magnesium ions (Randox, Crumlin, UK). HDL-C was isolated by centrifugation of the sample. The cholesterol content of the isolate was then determined using exactly the same method as described for the measurement of total cholesterol (section 3.5.4.1 (ii(g)(1)). The intensity of the produced colour was measured photometrically at 365 nm. A special control was used

as standard (Boehringer Mannheim-Roche Diagnosstic, Mannheim, Germany). Values are expressed in mmol/L. The C.V. for the method was 1.9%.

4. LDL cholesterol

LDL cholesterol (mmol/L) was determined using the following calculation (Randox, Crumlin, UK):

$$\text{LDL cholesterol} = \text{total cholesterol} - \frac{\text{triglycerides}}{2.2} - \text{HDL cholesterol}$$

Values were expressed in mmol/L. The C.V. for the method was 1.9%.

h. Serum total protein

Serum total protein (TP) was determined in duplicate on the Boehringer Mannheim Hitachi 902 chemistry analyser using a colourimetric method (cat. no. 1553 836, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany). Protein forms a coloured complex with cupric ions in alkaline medium. The intensity of the developed colour is proportional to the concentration of protein in the sample. The method was calibrated against the Calibrator for Automated Systems (cat. no. 759 350, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany). Precinorm U (normal values) (cat. no. 171 735, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany) and Precipath U (abnormal values) (cat. no. 171 760, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany) were used as control serum. The C.V. for the method was 1.0%.

i. Serum albumin

Serum albumin was determined in duplicate using the Bromocresol-green (BCG) method (cat. no. 1489 143, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany) on the Boehringer Mannheim Hitachi 902 chemistry analyser. Albumin

complexates with bromocresol-green at a pH of 4.2. The intensity of the coloured complex is directly proportional to the albumin concentration in the sample. This complex is measured with a spectrophotometer. The method was calibrated against the Calibrator for Automated Systems (cat. no. 759 350, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany). Precinorm U (normal values) (cat. no. 171 735, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany) and Precipath U (abnormal values) (cat. no. 171 760, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany) were used as control serum. The C.V. for the method was 1.5%.

j. Serum glucose

Serum glucose was determined in duplicate using an enzymatic colourimetric quantitative (GOD-PAP) method (cat. no. 1448 668) on the Boehringer Mannheim Hitachi 902 chemistry analyser. The method was based on oxidation of glucose to gluconic acid and hydrogen peroxide by glucose oxidase. Introduction of peroxidase and a chromogenic oxygen acceptor results in the formation of a colour that can be measured photometrically. The method was calibrated against the Calibrator for Automated Systems (cat. no. 759 350, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany). Precinorm U (normal values) (cat. no. 171 735, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany) and Precipath U (abnormal values) (cat. no. 171 760, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany) were used as control serum. The C.V. for the method was 1.3%.

k. Full blood count

The metabolic variables, haematocrit, haemoglobin, and the white and red blood cell counts were measured by means of a full blood count. The Coulter method was used. The Coulter method counts and sizes cells by detecting and measuring changes in electrical resistance when a particle in a conductive liquid passes through a small aperture.

1. White and Red blood cell count

Counting of red and white blood cells took place sequentially. First the system draws the white blood cell (WBC) dilution through the WBC aperture, then drains and rinses the bath. It then draws the red blood cell (RBC) dilution through the RBC aperture. The system counts both the RBC and WBC dilutions for three consecutive periods of 4s each. During the RBC count, pulses that represent cells as 36 fL or greater are classified as red cells. During the WBC count, pulses that represent cells as 35fL or greater are classified as white blood cells. The count cycle was monitored for abnormal variation using Coulter's Aperture Integrity Monitor (AIM). The C.V. for the WBC and RBC is 1.29% and 1.37% respectively.

2. Haemoglobin

The Coulter system uses the lysed white blood cell count dilution to measure haemoglobin concentrations. The absorbance of light from a Light Diode (LED) is measured at 525 nm through the optical pathlength of the bath. A beam of light from a Liquid Energy Display (LED) passes through the sample, through a 525 nm filter, and is measured by a photodiode. The signal is amplified and the voltage is measured and compared to the blank reference reading. The C.V. for haemoglobin and haematocrit is 1.96% and 2.01% respectively.

I. Plasma acetate

The acetic acid content of citrated plasma samples was measured in duplicate using the UV- method (cat. no. 148 261, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany). Acetic acid was converted in the presence of the enzyme acetyl-CoA synthetase (ACS) with adenosine-5'-triphosphate (ATP) and coenzyme A (CoA) to acetyl-CoA. The developing acetyl-CoA reacts with oxaloacetate to form citrate, in the presence of citrate synthase. The oxaloacetate required for the reaction is formed from malate and nicotinamide-adenine dinucleotide (NAD) in the presence of malate

dehydrogenase (MDH). In this reaction NAD was reduced to NADH. The determination is based on the formation of NADH measured by the increase in absorbance at 340nm. Since a preceding indicator reaction was used, the amount of NADH formed is not linearly proportional to the acetic acid concentration. A control sample (0.152 g/L) was supplied with the kit. The C.V. for the method was 1.9%.

3.5 Intervention

All subjects participating in the study were "standardised" by eating all their meals at the same mess. Individual variation in the diet was limited and could only have a small effect if any on the intervention.

3.5.1 Short chain fatty acid supplement

The SCFA supplement consists of sodium acetate (Merck NT Laboratory Supplies (Pty.) Limited, Hohenbrunn, Germany), sodium propionate (BDH Laboratory Supplies, England) and sodium butyrate (Merck NT Laboratory Supplies (Pty.) Limited, Hohenbrunn, Germany), approved for human use. The acetate, propionate and butyrate were given in a capsule in the following ratio 65:19:16, that is the same as the fermentation products of "oat bran" (Bugaut & Bentèjac, 1993). The capsules were enterically coated with a chelac-containing spray, ensuring the capsules to pass through the stomach and dissolve in the large gut to follow the same way as the SCFAs fermented from dietary fibre. Each capsule contained 500 mg of the SCFAs mixture. Each subject was supplied with 12 capsules, amounting to 6 g of SCFAs per day. This amount of SCFAs is equal to the amount of acids produced from the fermentation of approximately 15g of oats fibre (Wrong, 1995, p. 11). The capsules were a friendly gift from Quatromed, Bethlehem, SA.

3.5.2 Placebo

Maize starch (in the same capsule as the supplement) was used as a placebo. The capsules weighed 500 mg each. Each subject was supplied with 12 capsules per day.

3.6 FIELDWORKERS

Fieldworkers used in this study included:

- a qualified nurse at the SANDF who took the blood pressure of the subjects;
- four trained operational medical personnel of the SANDF who took the blood samples, and
- a trained primary health care worker of the SANDF who helped with the translation and the questionnaires where necessary.

The nurse and the operational medical personnel received training in the measurement of blood pressure, the drawing of blood samples as well as important factors to keep in mind when translating the questionnaires. The training of the fieldworkers was reliable and they were knowledgeable in the techniques used.

The researcher took the weight, height, waist and hip measurements of the subjects.

3.7 PILOT STUDY

The pilot study is a scaled down version of the large investigation.

The fieldworkers used in this study were trained during a pilot study. All the aspects involved in the collection of information as well as the procedure followed during the main study were explained to them.

The designed FFQ were executed on five members who did not take part in the main study. The kitchen manager also completed a FFQ to evaluate the portion sizes used

as well as the preparation of the foods, and the addition of food items. Food items not initially included in the questionnaire were also added to the questionnaire. Problems with understanding the information about the frequency needed from the FFQ were identified. These questions were stated very clearly during the study. Although the standard 24-hour recall method was used, it was also tested during the pilot study to standardise the method used to collect the information.

After the pilot study, the FFQ were coded and the normal portions sizes used in the mess were already included in the questionnaire.

3.8 MANAGEMENT

Sustained attention to the daily management and quality assurance of the study are essential (Dennis & Kris-Etherton, 1990, p.151). A strong management plan and capable investigative team are the key to avoiding problems that may be detrimental to the study. Components of a good management plan include organisation, communication, clear delineation and coverage of duties and responsibilities, contingency plans, and procedures for dealing with problems (Dennis & Kris-Etherton, 1990, p.151). The following management measures were taken to ensure that the goals of the study were met:

- The aim of the study, the study design, as well as all the practical arrangements involving the participating subjects were explained to the subjects' superior officers, who were also involved in the practical planning of the study. Superior officers were informed on the progress of the study, through frequent meetings at regular intervals during the course of the study.
    - The subjects were informed beforehand about the practical execution of the study.
    - The subjects were numerically arranged from 1 to 22 according to their force numbers.

- A placebo group was included in the study to determine the effect of other factors, such as seasonal changes, etc. on the measured metabolic variables.
- To make sure that fasting blood samples were taken, a late breakfast was arranged at the mess after the blood samples were drawn.
- The subjects ate at a reserved table at the mess during the course of the study.

- The Department of Biostatistics at the UOFS randomly divided the subjects into the supplement and control groups.
  - The capsules were counted beforehand by an outside individual participating neither in the project, nor in the execution of any facets of the project.
  - This ensured that both participants and researcher were blinded for the duration of the study.
  - The blind information was only made available after all results were supplied to the statistician in charge.
  - The capsules of the supplement and the placebo looked identical.
  - The researcher handed out the correct amount (4) of capsules and was present at each meal (breakfast, lunch and supper). If the researcher was not available, the kitchen manager handed out the capsules. Each subject's capsules were kept in his own bag, with his number clearly marked on it.
- It was of great importance that the subjects had to be continuously motivated throughout the study. The following measures were taken to keep the subjects motivated:
  - Informal social functions were arranged at regular intervals and accompanied by an afternoon's leave.
  - At the end of the study, the subjects received credit from their commanding officer in the form of a day's leave.
  - The subjects were followed-up daily at meal times. This helped with the evaluation of the progress of the study as well as with the participation of the subjects and also to identify any possible unwanted but inevitable problems.
  - At the end of the study, each subject received a certificate and a small gift of acknowledgement for taking part in the study.

- A good relationship of trust and confidentiality developed between the researcher and the subjects. This increased the reliability of the study.

- Only the researcher and the fieldworker were involved in the collection of data, which ensured that data were obtained in the most reliable and valid manner possible.

3.9 STATISTICAL ANALYSIS

Results are summarised by means, standard deviations (SD), medians, minimums, maximums (numerical variables) and frequencies and percentages (categorical variables). Changes within groups from baseline to follow-up are summarised by means and standard deviations with 95% confidence intervals. The changes in the two groups are compared using 95% confidence intervals (Gardner & Altman, 1989).
The confidence interval shows, the range, from the smallest to the largest values of that parameter that is consistent with the sample data. It is presented alone with the point estimated. The confidence interval provides additional information about magnitude that is useful for interpreting results.

Spearman rank correlations (with p-values) were calculated to determine the correlation between baseline values, as well as between changes from baseline to follow-up values.

The statistical analysis was done by the Department of Biostatistic, UOFS.

3.10 LIMITATIONS OF THE STUDY

The small sample size and over reporting of the dietary questionnaires were the two main limitations of the study.

Small sample size

Initially thirty subjects were planned for the sample size with 15 subjects in each group. After several attempts to find enough men for a big enough sample size, due to the inclusion criteria, the difficulty to find men that are non-smokers as well as the demanding program of the soldiers, it was only possible to identify 22 subjects to participate in the study. The sample size of 11 subjects in the experimental group and 10 subjects in the control group were still larger than the five to six subjectss reported in other studies on fibrin network structures using fresh plasma (Nair *et al*., 1991; Blombäck *et al*., 1992). Therefore the 21 subjects in this study was taken as sufficient to obtain meaningful results and therefore had no effect on the results of the study. However, because of the smaller sample size the results should be interpreted with caution.

One subject lost interest. He did not attend the meetings and was never available to complete the questionnaires. He was not included for the rest of the study and 21 subjects completed the study.

Over reporting

The dietary composition for the various dietary components was similar for the 24-hour recall and FFQ. The energy intake of the subjects according to the FFQ were, however, higher than the energy intake for the 24-hour recall. Therefore, can it be accepted that overestimated dietary intakes were reported with the FFQ (Appendix 8). The subject group was very homogeneous regarding their diet and ate all their meals at the mess and little if any daily variation occurred in the menu. Although the literature describes under-reporting for the 24-hour recall (Katzenellenbogen *et al*. 1999), the dietary composition of the subjects was very similar at baseline (Table 4.3) and at the end (Appendix 9) of the study. Therefore, the 24-hour recall can be taken as suitable to evaluate the usual dietary intake of the subjects in this study.

The second 24-hour recall taken at the end of the study was not of a typical day, due to a sports day held in the unit. Therefore only eleven 24-hour recalls could be analysed, and indicated that the dietary composition for the first and second 24-hour recalls was very similar. Furthermore, the fact that the menu used in the mess is pre-planned, supports the fact that there was little if any change in the usual eating pattern of the subjects.

Due to an unplanned field trip, some of the subjects did not receive their capsules for lunch on that specific day. Double the amount of capsules was taken at supper on that specific day. However, no side-effects were observed in any of these subjects.

3.10 SUMMARY

The design and successful implementation of a clinical trial are dependent on many factors, including careful planning and attention to all aspects of the study. This chapter discussed the study design, measurements chosen for the study and the data analysis to ensure that all the factors involved in a clinical trial were applied.

Very few problems occurred during the study and those that did were controlled and overcome. By strictly adhering to the rules of applying valid techniques and reliable precautionary measurements, the high standard of obtaining information in this study was carried out using reliable techniques and so provide valid information that clearly meets the pre-set gaols of the study.

CHAPTER 4

RESULTS

4.1 INTRODUCTION

The results of the study are presented in tables and graphs in this chapter, preceded by a short description of each. The aim is to describe the characteristics of the subject group in terms of coronary risk factors, haemostatic risk factors, and dietary intakes of the subjects of the experimental (SCFA) group and the control (Placebo) group at baseline and to indicate changes in these variables after the study period. Correlations between the baseline variables will also be shown, followed by a short summary.

4.2 BASELINE RESULTS

The first section of this chapter compares the baseline results between the experimental and placebo groups (Tables 4.1 - 4.4). The intention of this comparison is mainly to focus on the homogeneity of the two groups, as this may have been a determining factor in the outcome of the intervention trial.

4.2.1 Characteristics of the study group

4.2.1.1 Physical and geographical characteristics

It is evident from Table 4.1 that the subject population used for this study was of a homogeneous nature, which could mainly be ascribed to the strict inclusion criteria applied within the study. All the subjects used in the study were male and had a medium activity level (Table 4.1). The entire subject group was in their early to mid-twenties, except for one subject aged 34 years (Table 4.2). As indicated in Table 4.1, none of the subjects smoked, used any chronic medication or had any evidence of previous

Table 4.1 Characteristics of the SCFA and Placebo groups at baseline

VARIABLE	SCFAs	PLACEBO
Number of subjects	11	10
Sex	Male	Male
Activity level	Medium	Medium
Smoking	None	None
Cardiovascular events	No history	No history
Medication	None	None

cardiovascular events (CHD, stroke, etc.). None of the subjects had a family history of CHD or hypercholesterolaemia.

However, a family history of diabetes mellitus (1 subject in both groups) and hypertension (SCFA 4 subjects; Placebo 2 subjects) were present in both groups, while two subjects in the Placebo group had a family history of stroke.

The subjects were all members of the School of Armour Unit in Tempe, Bloemfontein, for at least one year, and were eating all their meals at the same mess.

4.2.1.2 Metabolic characteristics

Various metabolic indicators were used to compare the general health status of the two groups (Table 4.2).

Haemoglobin and haematocrit values were used as indicators for some possible metabolic disorders, such as iron deficiency, anaemia, liver disease, etc. (Gordon *et al*., 1974; Louann & Lawrence, 1996). The mean haematocrit (SCFA 45.5%; Placebo 46.1%) and haemoglobin (SCFA 15.5g/dL; Placebo 15.6 g/dL) concentrations compared well between the two groups, and were within the normal ranges.

The white and red blood cell counts, as well as the total protein, albumin and glucose concentrations were also used as indicators for the health status of the subjects (Table

Table 4.2 Age, metabolic indictors, coronary risk factors and haemostatic risk factors at baseline

	Normal ranges	SCFAs						PLACEBO					
		N	Mean	SD	Max	Med	Min	N	Mean	SD	Max	Med	Min
Age		11	23.7	3.77	34	23	20	10	23.4	1.9	26	23.5	21
Metabolic Indicators													
Haematocrit (%)	**41 – 52** [a]	11	45.5	3.0	52.8	45.1	41.9	10	46.1	3.1	51.0	46.8	41.3
Haemoglobin (g/dL)	**13.7 – 17.8** [a]	11	15.5	0.9	17.6	15.3	14.4	10	15.6	1.0	16.8	15.8	14.2
White blood cell count ($x10^3$/L)	2.53 – 8.43 [a]	11	6.7	3.6	13.9	4.7	3.1	10	6.1	1.2	7.9	5.9	4.5
Red blood cell count ($x10^6$/L)	4.49 – 5.90 [a]	11	5.3	0.6	6.5	5.3	4.6	10	5.3	0.5	6.5	5.1	4.8
Albumin (g/L)	34-52 [b]	11	53.0	3.4	58.0	52.0	47.0	9	53.3	3.5	59.0	55.0	47.0
Total Protein (g/L)	64-84 [b]	11	81.3	3.9	86.0	82.0	74.0	9	80.9	6.2	94.0	79.0	72.0
Glucose (mmol/L)	< 6.0 [c]	11	4.6	0.3	5.1	4.5	4.3	9	4.7	0.4	5.5	4.7	4.1
Coronary risk factors													
Anthropometry													
Weight (kg)		11	65.4	9.1	80.3	64.1	55.7	10	69.4	8.2	84.1	71.4	58.7
Height (m)		11	1.72	0.1	1.80	1.70	1.65	10	1.75	0.04	1.80	1.75	1.68
BMI (kg/m^2)	> 30 [d]	11	22.1	2.7	26.2	22.2	17.3	10	22.7	2.3	26.3	22.6	19.3
Waist (cm)		11	79.8	5.0	87.8	80.3	71	10	83.2	6.3	92.8	83.1	73.1
Hip (cm)		11	93.3	5.6	105.9	93.0	84.1	10	95.6	5.1	105	96.4	87.1
WHR	< 1.0 [e]	11	0.9	0.02	0.9	0.85	0.83	10	0.9	0.04	0.9	0.87	0.82
Blood Pressure													
SBP (mmHg)	< 160 [f]	11	126	6.7	140	130	120	10	131	8.8	140	130	120
DBP (mmHg)	< 90 [f]	11	85	8.2	100	80	70	10	86	9.7	100	85	70

Table 4.2 Age, metabolic indictors, coronary risk factors and haemostatic risk factors at baseline (continued)

	Normal ranges	SCFAs						PLACEBO					
		N	Mean	SD	Max	Med	Min	N	Mean	SD	Max	Med	Min
Lipid profile													
Total cholesterol (mmol/L)	3.0-5.2[g]	11	4.5	0.7	5.5	4.6	3.3	9	4.5	1.4	6.9	4.3	2.4
Triglycerides (mmol/L)	0.3-2.0[g]	11	0.8	0.3	1.5	0.7	0.5	9	1.1	0.4	2.0	0.9	0.7
HDL cholesterol (mmol/L)	0.9-1.6[g]	10	1.5	0.4	2.1	1.4	1.1	9	1.4	0.4	2.5	1.4	1.1
% HDL cholesterol* (%)	>20%	10	33.4	6	44.7	31.1	27.5	9	32.4	7.8	43.8	32.6	22.6
LDL cholesterol (mmol/L)	2.0 –3.4[g]	10	2.5	0.4	3.3	2.5	1.9	9	2.6	1.1	4.5	2.5	1.0
Haemostatic risk factors													
Fibrinogen (g/L)	1.8-3.5[h]	8	2.8	0.6	4.0	2.8	2.1	10	2.9	0.4	4.0	2.9	2.4
Fibrin monomers (mg/L)	**3.4 >< 14.5[h]**	7	4	0.6	4.6	3.9	3.3	10	4.1	0.8	5.6	3.9	3.2
Fibrin network content (g/L)		7	1.14	0.33	1.75	1.08	0.73	10	1.07	0.2	1.34	1.06	0.77
Compactions (%)		6	21.5	5.3	26.2	23.2	12.2	10	25.7	8.9	37.2	25.9	12.7
Mass to length ratio (μT)		7	48.7	15.2	73.1	44.3	30.3	10	56.8	15.3	92.6	55.2	41.3
FVII (%)	70-150%[h]	7	116.3	19.8	141.2	116.1	83.3	10	102.1	29.3	165.0	92.6	76.8
FVIII (%)	70-150%[h]	8	105.5	17.9	141.9	99.4	88.3	10	101.8	34.9	175.9	87.2	63.3
Acetate (μmol/L)	< 50[i]	10	39.6	25.8	91.0	39.0	8.0	10	26.3	17.8	54.0	30.0	3.0

* % HDL = HDL-cholesterol X 100
Total cholesterol

[a] Haematology Department, UOFS, 1997
[b] Chemical Pathology , UOFS
[c] SAIMR, 1991
[d] Steyn *et al*., 1990
[f] Steyn *et al*., 1991
[g] Institute for Pathology, University of Pretoria, 1999.
[h] Normal ranges provided by the manufacturer of the technique used
[i] Cummings *et al*., 1987a[e] Charlton *et al*., 1996

4.2). Both groups had a mean red blood cell count (SCFA & Placebo 5.3 $x10^{12}$/L), white blood cell count (SCFA 6.7 $x10^{9}$/L; Placebo 6.1 $x10^{9}$/L) and total protein levels (SCFA 81.3g/L; Placebo 80.9g/L) within the given reference values. The mean albumin levels (SCFA 53.0g/L; Placebo 53.3g/L) were, however, slightly higher than the given reference values for serum albumin. Normal fasting glucose levels were also evident in both groups (SCFA 4.6mmol/L; Placebo 4.7mmol/L).

As a whole it could be concluded that the subject group was of a homogeneous nature and had an apparently healthy clinical and physical appearance.

4.2.2 Coronary risk factors

The anthrompometry, blood pressure and lipid profile of the SCFA and Placebo groups were analysed at baseline to determine the presence of the known and accepted reversible risk factors, namely, overweight, hypertension, and dyslipidaemia.

4.2.2.1 Anthropometry

The average body weight (SCFA 65.4kg; Placebo 69.4kg) as well as height (SCFA 1.72m; Placebo 1.75m) of the Placebo group were slightly higher than those of the SCFA group at baseline (Table 4.2). The BMI of both groups (SCFA 22.1kg/$m^{2;}$ Placebo 22.7kg/m^{2}), however, was nearly similar, indicating normal weight among all the subjects. Although the waist (SCFA 79.8cm; Placebo 83.2cm) and hip (SCFA 93.3cm; Placebo 95.6cm) circumferences in the Placebo group were found to be slightly higher than the SCFA group, the WHR of 0.9 was measured in both groups, which was below the 0.1 cut-off point of android build (Charlton *et al.* 1996).

4.2.2.2 Blood pressure

The Blood pressure of the SCFA (126/85 mmHg) and Placebo (131/86 mmHg) groups was within normal ranges (<140/90mmHg), and there was no significant difference at baseline between these values of the two different groups (Table 4.2).

4.2.2.3 Lipid Profile

The results of the lipid variables namely, total cholesterol, LDL cholesterol, HDL cholesterol and serum triglycerides are summarised in Table 4.2.

The mean serum total cholesterol (4.5 mmol/L for both groups) and LDL cholesterol (SCFA 2.5mmol/L; Placebo 2.6mmol/L) of the two groups was within normal ranges. The total cholesterol levels were very comparable with other urban black populations in Qwa Qwa, Mangaung (Mollentze *et al*., 1995) and Durban (Seedat *et al*., 1992), and were higher than those of rural black South Africans (Jooste *et al*., 1990). Therefore, the higher normal total cholesterol levels found in the present study, show a trend towards Westernisation of this black population. Although the mean serum triglyceride levels were slightly higher for the Placebo group (1.1mmol/L) than for the SCFA group (0.8 mmol/L), these triglyceride values were still below the maximum cut-off (2.0 mmol/L) for healthy individuals. The difference between the two groups (0.3 mmol/L), however, is of no clinical significance. The mean HDL cholesterol levels of both groups were almost identical (SCFA 1.5 mmol/L; Placebo 1.4 mmol/L) and supports a favourable value. The % HDL cholesterol, which indicates the percentage of HDL from total cholesterol, was also nearly similar for the two groups.

It can be concluded that both subject groups used in this study had a favourable and nearly similar lipid profile, with a tendency of total cholesterol levels in the higher normal range.

4.2.3 Haemostatic risk factors

The haemostatic risk factors measured within this study included fibrinogen, factor VII activity, factor VIII activity and fibrin network architecture. These variables are presented in Table 4.2.

4.2.3.1 Fibrinogen

The mean fibrinogen levels (SCFA 2.8 g/L; Placebo 2.9g/L) were basically identical for both groups and fell within the normal fibrinogen ranges. The aim of this study was to investigate the possible effect of a SCFA supplement on fibrinogen levels in black men. The subject group was recruited by means of testing fibrinogen levels, as changeable fibrinogen levels were a prerequisite for inclusion in the study. A value of 2.8g/L lies above the average normal fibrinogen range (1.8 g/L and 3.5 g/L) (2.5g/L) and has the potential of a possible change in concentration.

4.2.3.2 Fibrin monomers

Fibrin monomers were measured in order to determine the concentration of soluble fibrin in plasma. Fibrin monomers are commonly used to diagnose hypercoagulable states. The mean fibrin monomer concentration was almost identical at baseline for both groups (SCFA 4.0 mg/L; Placebo 4.1 mg/L). This falls below the recommended cut-off point for fibrin monomer concentrations, as supplied by the manufacturer.

4.2.3.3 Factor VII activity

The SCFA group and the Placebo group had mean factor VII activity of 16.3 percent and 2.1 percent respectively, above the normal factor VII activity of 100 percent. The normal range is given as 50 percent above and 30 percent below the normal factor VII activity. It is evident from this that the factor VII activity levels for the SCFA group were higher than the normal ranges at baseline.

4.2.3.4 Factor VIII activity

Factor VIII activity was also below 150 percent the normal range in both groups, but both groups had factor VIII activities above the normal factor VIII activity of 100 percent. Although the SCFA group (105.5%) had slightly higher factor VIII activities than the Placebo group (101.8%), it was only a minor difference and not clinically significant.

4.2.3.5 C-Reactive protein

The CRP values were < 0.01g/L in both subject groups, which is an indication of the absence of any inflammatory disease. These CRP values were therefore not presented in Table 4.2.

4.2.3.6 Fibrin network architecture

The network fibrin content, compactions, mass length ratio from turbidity (μT) and lysis rates of fibrin networks are used to characterise the fibrin network at baseline (Table 4.2). The lysis rates of fibrin networks are presented in Figure 4.1 on p. 124.

The network fibrin content was similar in both groups (1.1 g/L). However, the compactions (SCFA 21.5%; Placebo 25.7%) and mass length ratio from turbidity (SCFA 48.7 Dal/cmx10^{12}; Placebo 56.8 Dal/cmx10^{12}) were higher in the Placebo group than in the SCFA group. The clinical significance of this is yet unknown.

4.2.4 Acetate

Acetate concentrations of the subjects were measured in this study due to the fact that the hypothesis stated that a SCFA supplement may influence fibrinogen concentrations as well as other haemostatic factors such as the fibrin network architecture. It is important to note that propionate and butyrate are almost completely metabolised by the liver and does not reach the systemic circulation (Cummings *et al*., 1987), making the

determination of these two fatty acids in systemic blood of no significance. Table 4.2 indicates that both the SCFA group and Placebo group had mean fasting acetate concentrations (39.6 μmol/L; 26.3 μmol/L, respectively) below 50 μmol/L as described by Cummings *et al*. (1987) for normal fasting acetate concentrations.

4.2.5 Dietary intake

The subjects ate all their meals at the mess, with 80 percent (SCFA 9; Placebo 8 subjects) eating three meals a day, and the other 20 percent (2 subjects) sometimes skipping breakfast. None of the subjects were on special diets but 5 subjects in the SCFA group and 6 subjects in the Placebo group indicated that they usually try to reduce their fat intake. The mean energy, macronutrient and micronutrient intake of both groups, obtained from the 24-hour recall at baseline, will be discussed and compared with the prudent guidelines and with the RDA component of the DRIs. The overestimated dietary intakes reported for the FFQ because of the similar macro- and micronutrient content between the 24-hour recall and FFQ but high energy intake reported for the FFQ, will therefore not be discussed, but the results are presented in Appendix 8. However, the dietary composition obtained from the FFQ compares well with the dietary composition obtained from the 24-hour recall.

4.2.5.1 Macronutrient intakes

The mean total energy for both groups compared well and was within the RDA for that specific age group (Table 4.3). The total fat intake (SCFA 29.5%; Placebo 26.4%) was nearly similar for both groups. Although the total fat intake was within the prudent guidelines, it was higher than the 16 percent reported for rural blacks (Jooste *et al*. 1990). The SFA (SCFA 8.2%, Placebo 7.6%), MUFA (SCFA 10.5%, Placebo 8.9%) and PUFA (SCFA 5.5%; Placebo 5%) compare well between the two groups. The SFA intake, however, was on the borderline, while the MUFA and PUFA were below the prudent recommendations. A relatively high P/S ratio was reported for both groups

Table 4.3 Mean daily energy, macronutrient and micronutrient intake according to the 24-hour recall within the SCFA group and Placebo group

	RECOMMENDATIONS	SCFAs						PLACEBO					
		N	Mean	SD	Max	Med	Min	N	Mean	SD	Max	Med	Min
	Prudent Guidelines												
Total Kilojoules (KJ)	9660-12 600	11	10014.6	2737.7	13758.2	9013.3	5500.8	10	9623.7	3558.2	17118.8	8174.4	6003.1
Total Protein (g)		11	95.2	21.1	121.6	95.5	51.9	10	103.8	33.4	156.8	100.9	41.1
Total Protein (%E)	*12-20% TE*	*11*	*16.9*	*4.3*	*24.9*	*16.2*	*10.3*	*10*	*18.6*	*3.3*	*22.6*	*18.8*	*11.6*
Plant protein (g)		11	32.8	10.2	51.5	31.6	18.4	10	33.6	17.5	71.6	28.5	15.8
Plant protein (%E)	50% TP	*11*	*5.6*	*0.8*	*6.9*	*5.6*	*4.4*	*10*	*5.8*	*1.4*	*7.6*	*6.1*	*3.8.*
Animal protein (g)		11	62.6	17.9	84.9	65.8	26.7	10	70.2	23.2	99.1	82.7	14.3
Animal protein (%E)	50% TP	*11*	*11.3*	*4.3*	*19.3*	*11.8*	*5.3*	*10*	*12.8*	*4.1*	*17.2*	*14.2*	*4*
Total Fat (g)		11	78.6	31.6	135.1	73.3	37.3	10	68.3	30.7	121.2	59.9	28.9
Total Fat (%E)	*< 30% TE*	*11*	*29.5*	*42.1*	*42.1*	*30.6*	*17.3*	*10*	*26.4*	*5.9*	*34.6*	*26.1*	*18.1*
SFA (g)		11	21.7	10.1	40.5	16.7	8.5	10	19.3	9.7	32.5	19.8	6.2
SFA (%E)	< 8% TE	*11*	*8.2*	*2.6*	*12.5*	*8.1*	*3.9*	*10*	*7.6*	*3.1*	*12.4*	*7.1*	*3.8*
MUFA (g)		11	28.2	14.1	52.8	23.9	10.3	10	22.9	11.9	42	20.1	10.2
MUFA (%E)	>12% TE	*11*	*10.5*	*3.8*	*16.5*	*10.7*	*4.8*	*10*	*8.9*	*2.9*	*13.6*	*8.4*	*4.9*
PUFA (g)		11	14.3	7.4	28.8	15.3	5.0	10	13.3	8.7	31.4	10.9	5.5
PUFA (%E)	10% TE	*11*	*5.5*	*2.9*	*12.8*	*4.9*	*2.1*	*10*	*5*	*1.9*	*8.3*	*5.3*	*2.5*
P/S ratio		11	0.7	0.4	2.0	0.6	0.3	10	0.8	0.5	1.7	0.6	0.3
Cholesterol (mg)	< 300	11	306.1	113.4	450.7	285.4	129.2	10	427.3	168.7	743.7	433.4	205.6
Total Carbohydrates (g)		11	300.9	98.3	461.5	300.8	147.6	10	290.3	109.9	541.5	248.2	187.3
Total Carbohydrates (%E)	> 50% TE	*11*	*50.7*	*8.2*	*62.2*	*50.5*	*38.2*	*10*	*51.7*	*6.8*	*67.3*	*51.4*	*42.5*
Added Sugar (g)		11	60.1	33.6	107	68.5	3.8	10	58.1	21.5	102.1	50.9	33.6
Added Sugar (%E)	< 15% TE	*11*	*12.4*	*6.7*	*21.5*	*12.8*	*1.6*	*10*	*11.1*	*3.7*	*15.7*	*11.9*	*5.5*

Table 4.3 (continued) Mean daily energy, macronutrient and micronutrient intake according to the 24-hour recall within the SCFA group and Placebo group

	RECOMMENDATIONS	SCFAs						PLACEBO					
		N	Mean	SD	Max	Med	Min	N	Mean	SD	Max	Med	Min
Alcohol (g)	< 5% TE	11	0	0	0	0	0	10	0	0	0	0	0
Total dietary fibre (g)	20-30	11	22.1	9.2	39.2	24.2	8.9	10	23.7	13.9	55.2	17.9	11.6
Total NSP (g)		11	3.9	2.8	8.7	4.2	0	10	4.2	4.4	15.2	2.8	0
Micronutrients	**RDAs**												
Vitamin A (μg RE)[a]	1000	11	1012.1	1393.1	4566.2	448	131.5	10	1145.1	2.9	7678.5	438.9	123.6
Vitamin C (mg)	60	11	114.8	108	280.6	51.6	8.6	10	92.6	62.7	172.2	105.1	9.2
Vitamin E (mg)	10	11	7.8	6	24.5	7.8	1.6	10	8.0	3.9	15.3	9.1	1.8
Vitamin D (μg)	5	11	3.9	2.6	7.6	4.7	0.8	10	6.1	4.6	14.2	6.6	0.1
Thiamine (mg)	1.2	11	1.3	0.8	2.4	1.3	0.5	10	1.3	0.7	3.0	1.0	0.7
Riboflavin (mg)	1.3	11	1.9	0.9	3.5	1.9	0.8	10	1.9	0.9	3.9	1.8	0.7
Niacin (mg)	16	11	24.9	9.4	37.7	27.7	6.2	10	24.0	9.2	35.7	22.6	4.2
Vitamin B6 (mg)	1.3	11	1.7	0.6	2.4	1.9	0.5	10	1.6	0.8	3.1	1.5	0.3
Vitamin B12 (μg)	2.4	11	5.4	2.2	9.6	5.6	2.5	10	6.6	2.9	9.9	7.4	2.1
Calcium (mg)	1000	11	811.4	391.7	1353.6	785.1	125.6	10	802.1	434.4	1741.5	697.7	274.0
Magnesium (mg)	400	11	350.9	113.7	540.5	368.9	173.2	10	381.8	181.2	795.8	316.9	225.3
Phosphorus (mg)	700	11	1370.3	383.6	1962.7	1319.7	792	10	1535.9	673.0	2860.2	1325.5	656.4
Iron (mg)	10	11	16.0	12.0	48.5	13.9	6.0	10	16.1	9.0	38.3	12.6	7.2

RDA: Recommended Dietary Allowance as a component of the RDIs
%TE: Values expressed as percentage of total daily energy intake.
%TP: Values expressed as percentage of total protein
P/S: Polyunsaturated/saturated fatty acids
[a] RE: Retinol equivalents. 1 retinol equivalent = 1 μg retinol

(SCFA 0.7; Placebo 0.8). The imprudent fat intake of the subjects, as well as the high cholesterol intake (SCFA 306.1mg; Placebo 427.3mg) in both groups show a tendency towards the adoption of a westernised diet as it compares well with other urbanised black populations in South Africa (Bourne e*t al*., 1993; Silvis & Mollentze, 1995; Slabber *et al*., 1997).

Dietary protein intake compares well between the two groups and contributed approximately 17 percent of total daily energy intake for both groups. It was mainly of animal origin (67% of total protein). Although these dietary protein intakes were still within the prudent guidelines, they were higher than the total protein and animal protein intakes of other urban black populations in South Africa (Bourne *et al*., 1993; Silvis & Mollentze, 1995; Slabber *et al*., 1997). The plant and animal proteins were approximately equal in rural African populations (Silvis & Mollentze, 1995), but higher animal protein intakes were reported for urban black populations (Slabber *et al.,* 1997). This high animal and total protein intake can therefore also be seen as a trend towards a Westernised diet.

Although total available carbohydrate intake was within the prudent guidelines of 50 percent or more of total energy, it was on the low range (SCFA 50.7%; Placebo 51.7%) in both groups. Total carbohydrate in the present study was extremely lower than the 62 to 70 reported for urban blacks in the 70's (Manning *et al*., 1974). Reported added sugar intake was within the recommended 15 percent of total energy, ranging from 12.4 percent in the SCFA group to 11.05 percent in the Placebo group. The added sugar intake of the present study group compares with the added sugar intake of the BRISK population, but were higher than the added sugar intakes of black populations in the Free State (Silvis & Mollentze, 1995; Slabber, *et al*., 1997). The mean dietary fibre intake was 22.1g and 23.7g for the SCFA group and Placebo group, respectively, which was markedly lower than the 48g reported for rural populations (Jooste *et al*., 1990). Alcohol abusers were not included in the study according to the inclusion criteria and therefore no alcohol consumption was reported in either of the groups.

All the signs for a tendency towards the adoptions of an atherogenic Westernised diet were mirrored in the present study group, namely:

- Fat intake in the higher normal range, with high SFA and cholesterol as well as low MUFA and PUFA intake compared to the prudent guidelines;
- high intake of total protein, mainly from animal origin;
- total carbohydrate and dietary fibre intakes on the lower range of the prudent guidelines with an increased intake of added sugar.

It can therefore be concluded that the usual dietary pattern of the present study group reflects a transitional phase towards a Western diet when compared with other rural and urban black populations in South Africa.

Although overestimated intakes of the different food items were reported in the FFQ, the dietary composition of this questionnaire supports the adoption of a westernised eating pattern (Appendix 8). Total protein contributed approximately 15 percent of total energy, comprised mainly of animal protein, and high (approximately 36%) total fat, high (> 300 mg) cholesterol (SCFA 565.4mg; Placebo 656.9mg), and low (<50% of total energy) total carbohydrate intakes were also reported according to the FFQ.

4.2.5.2 Micronutrient intake

The micronutrient intakes compare well between the SCFA and Placebo groups (Table 4.3). Vitamin and mineral intakes tended to meet the RDAs. Although SA food tables for vitamin D are not completed and therefore not reliable regarding vitamin D were intakes below the currently available RDAs reported for vitamin D in the SCFA group (3.9mg), with high intakes in the Placebo group (6.1mg). Mean vitamin E, calcium, and magnesium intakes below the RDA were reported for both groups.

4.3 INTERVENTION

Baseline results were compared with results obtained after a five-week dietary supplementation phase. The end results are not shown in this chapter, but the mean differences for each group between the baseline and end results are presented. Some of the subjects' biochemical variables could not be analysed. To evaluate the effects of the intervention, it was necessary to use only those subjects whose biochemical values were available before and after the intervention. Those variables where a different baseline value was obtained after exclusion of the subjects where the biochemical analysis was not possible are shown in Table 4.4. Compared to the baseline results presented in Table 4.2 this difference is very small and therefore of no significance.

4.3.1 Baseline results used for intervention

To describe the effect of SCFA supplementation, the same baseline results for all the variables (Table 4.2), except for those indicated in Table 4.4, were used. The only difference was in the haemoglobin, haematocrit, white and red blood cell count, and acetate of only the placebo group (Table 4.4).

Table 4.4 Baseline results used for the interpretation of those variables that changed for the intervention study results

	Placebo					
	N	**Mean**	**SD**	**Max**	**Med**	**Min**
Haemoglobin (g/dL)	8	15.5	1.1	16.8	15.3	14.2
Haematocrit (%)	8	45.8	3.5	51	46.2	41.3
White blood cell count ($x10^3$/L)	8	6.31	1.22	7.9	6.35	4.7
Red blood cell count ($x10^6$/L)	8	5.32	0.58	6.47	5.05	4.81
Acetate (μmol/L)	9	28.89	16.69	54.0	31.0	7.0

4.3.2 Dietary change and tolerance of supplement

The 24-hour recall taken at the end of the study indicated that there were very few variations in the usual diet of the subjects (Appendix 9). The dietary composition was very similar at baseline and at the end of the study. No change in the usual diet of the subject indicates that the results obtained after the intervention could be ascribed to the effect of the supplement and not to any dietary changes.

The supplements were tolerated very well. Except for the inconvenient amount of capsules consumed with each meal (4 capsules with each meal), no side-effects were present among the subjects. A total amount of 12 capsules were consumed daily. Two subjects in both groups experienced a decrease in appetite, while one subject in the SCFA group and four subjects in the Placebo group experienced an increase in appetite.

4.3.3 Intervention results

The differences between baseline and end results within the SCFA and Placebo groups respectively, as well as the mean difference in the variables between the two groups will be described according to metabolic indicators, coronary risk factors, haemostatic risk factors and acetate.

4.3.3.1 Metabolic Indicators

Mean differences between baseline and end results for the metabolic indicators used to describe the general health status of the two groups are presented in Table 4.5. There was a statistically significant increase in the haemoglobin and haematocrit concentrations of the SCFA group. Although a significant increase was observed in the haemoglobin and haematocrit values in the SCFA group, these values were still within the normal range for black men. The increase can therefore be taken as clinically non-significant. The red blood cell count within the SCFA group increased, while the white

Table 4.5 Mean difference within variables from baseline to end of intervention. The mean difference between the Placebo and supplementation groups at the end of the intervention is also supplied.

	SCFA				PLACEBO				Mean difference	95% CI# between groups
	N	Mean	SD	95% CI#	N	Mean	SD	95% CI#		
Metabolic indicators										
Haematokrit (%)	11	2.21*	2.70	0.39; 4.02	8	0.39	3.36	-2.42; 3.19	1.82	-1.11; 4.75
Haemoglobin (g/dL)	11	0.74*	0.93	0.11; 1.36	8	0.39	1.11	-0.54; 1.31	0.35	-0.64; 1.34
White blood cell count ($x10^3$/L)	11	-0.22	2.20	-1.69; 1.26	8	-0.83*	0.98	-1.65; -0.004	0.61	-1.16; 2.37
Red blood cell count ($x10^6$/L)	11	0.26*	0.38	0.007; 0.51	8	-0.02	0.41	-0.36; 0.33	0.28	-0.11; 0.66
Albumin (g/L)	11	0.45	2.54	-1.25; 2.16	9	1.89	3.69	-0.95; 4.72	-1.43	-4.37; 1.49
Total Protein (g/L)	11	1.36	3.78	-1.17; 3.89	9	3.78	7.16	-1.72; 9.28	-2.41	-7.64; 2.82
Glucose (mmol/L)	11	-0.66*	0.47	-0.97; -0.34	9	-0.84*	0.42	-1.17; -0.52	0.19	-0.24; 0.61
Acetate (μmol/L)	10	62.9*	65.21	16.25; 109.55	9	14.67	21.8	-2.09; 31.42	48.23	0.002; 96.46ə
Coronary risk factors										
Anthropometry										
Weight (kg)	11	-0.46	2.01	-1.81; 0.89	10	-0.49	1.43	-1.51; 0.53	0.03	-1.58; 1.63
BMI (kg/m^2)	11	-0.16	0.67	-0.61; 0.28	10	-0.16	0.46	-0.49; 0.17	-0.003	-0.53; 0.52
WHR	11	-0.001	0.007	-0.006; 0.004	10	-0.002	0.005	-0.006; 0.001	0.0006	-0.005; 0.006
Lipid Profile										
Total cholesterol (mmol/L)	11	0.14	0.39	-0.12; 0.39	9	0.24	0.78	-0.36; 0.84	-0.11	-0.67; 0.45
Triglycerides (mmol/L)	11	0.14	0.32	-0.08; 0.36	9	0.06	0.63	-0.43; 0.54	0.08	-0.38; 0.54
HDL cholesterol (mmol/L)	10	0.12*	0.15	0.006; 0.202	9	0	0.175	-0.13; 0.13	0.12	-0.04; 0.27
% HDL cholesterol	10	1.56	4.47	-1.6; 4.76	9	-1.91	6.09	-6.59; 2.77	3.47	-1.67; 8.61
LDL cholesterol (mmol/L)	10	-0.013	0.45	-0.33; 0.31	9	0.22	0.64	-0.28; 0.71	-0.23	-0.76; 0.30

Table 4.5 (continued) **Mean difference within variables from baseline to end of intervention. The mean difference between the Placebo and supplementation groups at the end of the intervention is also supplied.**

	SCFA				PLACEBO				Mean difference	95% CI# between groups
	N	Mean	SD	95% CI#	N	Mean	SD	95% CI#		
Haemostatic risk factors										
Fibrinogen (g/L)	8	0.1	0.28	-0.14; 0.34	10	0.03	0.79	-0.54; 0.59	0.07	-056; 0.69
Fibrin monomers (mg/L)	7	-0.44*	0.44	-0.85; -0.04	10	0.24	0.78	-0.32; 0.78	-0.68	-1.38; 0.02
Network fibrin content (g/L)	7	-0.24*	0.22	-0.44; -0.03	10	-0.13	0.37	-0.39; 0.13	-0.11	-0.44; 0.22
Compactions (%)	6	18.6*	8.76	9.48; 27.88	10	2.36	8.46	-3.67; 8.41	16.32	6.84; 25.81ɔ
Mass to length ratio (μ_T)	7	0.66	12.62	-11.01; 12.33	10	-4.89	13.53	-14.57; 4.79	5.55	-8.28; 19.38
Factor VII (%)	7	-13.47*	13.69	-26.13; -0.81	10	3.71	24.88	-14.08; 21.50	-17.18	-39.37; 5.0
Factor VIII (%)	8	-18.54*	13.59	-29.90; -7.17	10	-4.53	35.84	-30.17; 21.11	-14.01	-42.51; 14.49

\# 95 % CI = 95 % Confidence interval
* Significantly different between baseline and end results within the groups
ɔ Significant difference in baseline and end results between the SCFA group and Placebo group

blood cell count within the Placebo group decreased significantly. Fasting glucose levels were also significantly lower in both groups. As this change in fasting glucose levels occurred in both groups, it can be assumed that these changes could be due to other external factors and not to the supplement.

4.3.3.2 Coronary risk factors

No significant change in any of the anthropometric measurements occurred within either of the subject groups or between the subject groups (Table 4.5). The lipid profile did not change much either within or between the groups. A significant increase, however, was indicated by the 95 percent confidence interval in HDL cholesterol concentrations in the SCFA supplemented group. It is important to note that there was no statistically significant change in the % HDL concentrations. The SCFA supplemented group, however, tended to cause a small non-significant increase in the % HDL-values, as in contrast, the % HDL cholesterol values showed a small, non-significant decrease in the Placebo group. However, the non-significant increase in the % HDL cholesterol, renders the increase in HDL cholesterol in the SCFA group of no clinical significance.

4.3.3.3 Haemostatic risk factors

No statistical significant change was evident in fibrinogen levels in either of the two groups. However, a significant decrease (indicated by the 95% confidence interval) in factor VII activity, factor VIII activity, fibrin monomers and network fibrin content, as well as a significant increase in the compactions of the networks were measured in the SCFA supplemented group. No significant changes in the haemostatic risk factors were present in the Placebo group. Comparing the changes in the SCFA group and Placebo group, a statistical significant change was found in the compactions of the networks. Although no significant change was present in the mass to length ratio of the fibrin networks (μ_T) of the SCFA group, changes when comparing the two groups, indicate a more favourable effect of the SCFA supplement on the mass to length ratio (μ_T) of the fibrin networks. These results are supported by the lysis rates obtained from both subject groups. Fig 4.1 indicates

Fig. 4.1 Lysis rates of the SCFA group at baseline and at the end of the study

Fig. 4.2 Lysis rates of the Placebo group at baseline and at the end of the study.

that the SCFA supplement caused an increase in the network lysis rates when compared with the results obtained from the Placebo group (Fig. 4.2).

Although statistical significant differences were observed within the two groups were no statistical significant changes observed between the two groups. This could be due to the small study sample causing wide variation in the confidence intervals. A bigger study sample will possibly lead to more reliable differences between the two groups.

4.3.2.4 Acetate

Acetate concentrations increased significantly from baseline towards the end of the study in the SCFA group. Furthermore, the significant mean difference in acetate concentrations between the two groups supports the assumption that the rise in fasting acetate concentrations in the SCFA supplemented group was large enough for clinical effects on the measured variables.

4.4 CORRELATIONS

Only significant correlations between baseline variable of the subject group as a whole, as well as the significant correlations between changes from baseline to the end of intervention for the SCFA group and placebo group respectively, are presented. Some of the important haemostatic correlations with other variables and other striking significant correlations will be highlighted.

4.4.1 Correlation between baseline results of the subject group as a whole

Significant correlations between the baseline results of the subject group as a whole are presented in Table 4.6. A significant positive correlation between plasma fibrinogen concentrations and network fibrin content and fibrin monomer concentrations was found at baseline within the group as a whole. This significant correlation possibly indicates an association between the insoluble fibrin formed from the soluble fibrin monomers due to the action of thrombin on fibrinogen.

Compactions positively correlated significantly with μ_T, indicating that the tensile strength of the fibres correlates with the thickness of the fibres. Acetate concentrations also correlated positively with fibrin monomer concentrations. A negative correlation between network fibrin content and body weight was also prevalent.

Table 4.6 Correlation between baseline results for the subject group as a whole

VARIABLE 1	VARIABLE 2	N	Correlation coefficient	P-value
Weight	BMI	21	0.91	0.00
Diastolic blood pressure	Systolic Blood pressure	21	0.84	0.00
Total Cholesterol	Albumin	20	0.66	0.00
	Total protein	20	0.55	0.01
	HDL cholesterol	19	0.66	0.00
	LDL cholesterol	19	0.92	0.00
LDL cholesterol	% HDL cholesterol	19	-0.53	0.02
HDL cholesterol	% HDL cholesterol	19	0.47	0.04
Fibrinogen	Network fibrin content	17	0.64	0.01
	Fibrin monomers	17	0.56	0.02
Network Fibrin content	Weight	17	-0.48	0.05
Compactions	μ_T	16	0.58	0.02
Haematokrit	Haemoglobin	21	0.91	0.00
	Red blood cell count	21	0.72	0.00
	Albumin	20	0.5	0.03
	Total Protein	20	0.53	0.02
Haemoglobin	Albumin	20	0.45	0.05
	Total Protein	20	0.46	0.04
White blood cell count	Albumin	20	0.47	0.04
Red blood cell count	Total protein	20	0.56	0.01
Albumin	Total protein	20	0.67	0.00
	Triglycerides	20	0.64	0.00
	LDL cholesterol	19	0.82	0.00
	% HDL cholesterol	19	-0.45	0.05
Total Protein	LDL cholesterol	19	0.71	0.00
Acetate	Fibrin monomers	7	0.79	0.03

4.4.2 Correlation between changes from baseline to end for the SCFA supplemented group

Significant correlations between changes from baseline to end of intervention in the SCFA supplemented group are presented in Table 4.7. The change in factor VII activity from baseline to end of supplementation correlated significantly with the changes in factor VIII activity (negatively), network fibrin content (positively), haemoglobin concentrations, haematocrit and the red blood cell count. Changes in factor VII activity correlated negatively, and changes in factor VII activity correlated positively, with changes in albumin levels. The changes in network fibrin content correlated positively with red blood cell count.

The changes in compactions and μ_T from baseline to end correlated positively with the changes in total cholesterol, and changes in compactions also correlated significantly with the changes in LDL cholesterol concentrations. This could possibly indicate that SCFA supplementation has an effect on the lipid profile through an alteration in the fibrin network structure. Changes in fasting glucose concentrations correlated negatively with changes in HDL cholesterol as well as changes in the fibrin monomer concentrations. Changes in acetate concentrations from baseline to the end of supplementation correlated significantly and positively with the changes in fibrin monomer concentrations, indicating that the alteration in the characteristics of the fibrin networks could possibly have a direct effect of SCFA supplementation.

Table 4.7 Correlation between changes from baseline to end for the SCFA supplemented group

VARIABLE 1	VARIABLE 2	N	Correlation coefficient	P-value
Weight	BMI	11	0.99	0.00
	Total Protein	11	-0.66	0.03
BMI	Total Protein	11	-0.63	0.04
WHR	Total Cholesterol	11	0.62	0.04
	Glucose	11	-0.76	0.01
Diastolic blood pressure	Systolic Blood pressure	11	0.87	0.00
Total Cholesterol	Total protein	11	0.63	0.04
	LDL cholesterol	10	0.71	0.02

Table 4.7 Correlation between changes from baseline to end for the SCFA supplemented (continued) group

VARIABLE 1	VARIABLE 2	N	Correlation coefficient	P-value
HDL cholesterol	% HDL cholesterol	10	0.87	0.00
Factor VII	Factor VIII	7	-0.75	0.05
	Network fibrin content	7	0.86	0.01
	Red blood cell count	7	0.82	0.02
	Albumin	7	0.85	0.02
	Haemoglobin	7	0.87	0.01
	Haematocrit	7	0.82	0.02
Factor VIII	Albumin	8	-0.71	0.05
Fibrin monomers	Glucose	7	-0.75	0.05
	Acetate	7	0.79	0.03
Network fibrin content	Red blood cell count	7	0.86	0.01
	Haematocrit	7	0.86	0.01
	Haemoglobin	7	0.79	0.04
Compactions	Total cholesterol	6	0.81	0.05
	LDL cholesterol	5	0.97	0.01
μ_T	Total cholesterol	7	0.77	0.04
Haematokrit	Haemoglobin	11	0.92	0.00
	Red blood cell count	11	0.74	0.01
	Albumin	11	0.92	0.00
	Total Protein	11	0.80	0.00
	LDL cholesterol	10	0.79	0.01
Haemoglobin	Red blood cell count	11	0.67	0.02
	Albumin	11	0.87	0.00
	Total Protein	11	0.68	0.02
White blood cell count	Triglycerides	11	-0.62	0.04
Red blood cell count	Albumin	11	0.62	0.04
Total Protein	LDL cholesterol	10	0.87	0.00
Glucose	HDL cholesterol	10	-0.68	0.03
Acetate	Weight	10	0.63	0.05

4.4.3 Correlation between changes from baseline to end for the Placebo group

Significant correlations between the changes from baseline to end in the Placebo group are presented in Table 4.8. The changes in the mass to length ratio of fibrin fibres in networks (μ_T) correlated significantly with changes in weight, BMI, systolic blood pressure and acetate concentrations in the Placebo group. Changes in the fibrin monomer concentrations correlated with the changes in weight in this group. The change in total cholesterol from baseline to the end of the intervention correlated positively with the changes in glucose levels and negatively with the changes in HDL cholesterol concentrations. The significant correlation between the changes in the network fibrin content and changes in the fibrinogen concentration in the placebo group indicates that there is an association insoluble fibrin formed from plasma fibrinogen concentrations. This is also seen in the baseline correlations. A significant negative correlation was also found between the changes in compaction and changes in factor VII activity and acetate concentrations in the Placebo group.

Table 4.8 Correlation between changes from baseline to end for the Placebo group

VARIABLE 1	VARIABLE 2	N	Correlation coefficient	P-value
Diastolic blood pressure	Systolic Blood pressure	10	0.73	0.02
Total Cholesterol	Glucose	9	-0.70	0.04
	LDL cholesterol	9	0.94	0.00
	% HDL cholesterol	9	-0.71	0.03
LDL cholesterol	% HDL cholesterol	9	-0.75	0.02
Fibrinogen	Network fibrin content	10	0.67	0.03
Fibrin monomers	WHR	10	-0.66	0.04
Mass to length ratio (μ_T)	Acetate	9	0.68	0.04
	Weight	10	0.67	0.04
	BMI	10	0.72	0.02
	Systolic blood pressure	10	0.82	0.00
Compactions	Factor VII	10	-0.73	0.02
	Acetate	9	-0.7	0.04

Table 4.8 Correlation between changes from baseline to end for the Placebo group (continued)

VARIABLE 1	VARIABLE 2	N	Correlation coefficient	P-value
Haematokrit	Haemoglobin	8	0.93	0.00
	Red blood cell count	8	0.99	0.00
	Total Protein	7	0.99	0.00
	% HDL cholesterol	7	-0.82	0.02
Haemoglobin	Red blood cell count	8	0.96	0.00
	Albumin	7	0.96	0.00
	Total Protein	7	0.96	0.00
	% HDL cholesterol	7	-0.86	0.01
Red blood cell count	Albumin	7	1.0	0.00
	Total protein	7	0.99	0.00
	% HDL cholesterol	7	-0.82	0.02
Albumin	Total protein	9	0.91	0.00
	% HDL cholesterol	9	-0.83	0.01
Total Protein	% HDL cholesterol	9	-0.67	0.05
Acetate	Weight	9	0.68	0.05
	BMI	9	0.73	0.03

4.7 SUMMARY

The baseline results presented in this chapter indicated that the middle aged, black men used for this intervention trial were a homogeneous study group. There was no evidence of dyslipidaemia or any other known coronary and haemostatic risk factors, except for higher normal total cholesterol levels and factor VII and factor VIII activities in both groups. The trend towards the adoption of a high-fat, low-carbohydrate and low-fibre atherogenic westernised diet was also evident in these urbanised black men. After supplementation of five weeks, it was possible to indicate a significant increase in the HDL cholesterol concentrations as well as a significant decrease in factor VII activity, factor VIII activity, fibrin monomers and the network fibrin content for the SCFA supplemented group. The characteristics of the fibrin network architecture were also beneficially changed after SCFA supplementation. This can be seen in the significant increase of the compactions of

the network as the tendency of the mass to length ratio of the fibrin fibres to increase. No changes were evident, however, in the fibrinogen concentration in both groups. A significant increase was also seen in the fasting acetate concentration in the SCFA supplemented group. Possibly the most striking significant correlation found in this study indicates that after SCFA supplementation the network fibrin content and fibrin monomer concentrations decreased, while plasma fibrinogen concentrations did not change. Furthermore, this decrease in fibrin monomer concentrations positively correlated with the increase in acetate concentrations in the SCFA supplemented group.

CHAPTER 5

DISCUSSION

5.1 INTRODUCTION0

The aim of the study was to determine the effect of a combination of SCFAs on plasma fibrinogen concentrations, other haemostatic risk factors and coronary risk factors. Baseline results will be briefly discussed to evaluate the risk profiles of this apparently healthy study group. The effects of the supplement were measured against a placebo group, in order to examine whether SCFA supplementation (acetate:propionate: butyrate – 65:19:16) could possibly play a role in the treatment and prevention of CHD and stroke. The most important changes caused by the SCFA supplement that will be discussed include:

- significant increase in HDL concentration, although not clinically significant;
- significant decrease in factor VII, factor VIII activities, fibrin monomer concentration, and network fibrin content;
- significant increase in compactions of fibrin networks and mass to length ratio of fibrin fibres; and
- significant increase in fasting acetate concentrations.

The most prevalent correlations between the relevant variables will also be considered in this discussion. These findings will be interpreted in the light of findings from other relevant studies.

5.2 BASELINE RESULTS

As shown in chapter 4, when comparing the baseline results between the experimental and placebo groups, it was clear that this study group was of a homogeneous nature and can be seen as the perfect group to serve the purpose of the study. The study group as a whole had a healthy clinical and physical appearance, as well as a favourable metabolic profile. The study group also had a

favourable lipid profile; however, their total cholesterol levels showed a tendency towards the higher normal range. Similar lipid profiles with a tendency towards higher normal total cholesterol levels were found in urban black populations in the Free State (Mollentze *et al*., 1995; Slabber *et al*., 1997). In contrast, favourable lipid profiles together with total cholesterol levels in the lower normal range were described in other urban black populations in South Africa (Steyn *et al*., 1991; Vermaak *et al*., 1991; Oelofse *et al*., 1996).

Although higher normal plasma fibrinogen levels were a prerequisite for inclusion in the present study, the mean factor VII activity and factor VIII activity of the subjects were also in the higher normal range at baseline for the present study. In this context, it is important to note that Cushman *et al*. (1996) demonstrated an association between fibrinogen, factor VII and factor VIII activities and stroke.

Fibrinogen, fibrin monomer concentrations and the network fibrin content positively correlated significantly at baseline within the subject group as a whole. This correlation possibly indicates that when thrombin and fibrinogen interact, and fibrin monomers are generated to form insoluble fibrin networks, the concentrations of fibrinogen, fibrin monomers and fibrin fibres formed are in relationship with each other. This correlation has never been shown by any other available study. However, this correlation holds important implications for further research concerned with haemostatic risk factors, as it may be used to explain some effects on haemostasis not indicated by the measurement of plasma fibrinogen concentration alone.

The usual dietary intake of the subjects shows a tendency towards the adoption of an atherogenic westernised diet. The dietary composition of the subjects was within or on the borderline of the recommended prudent guidelines and includes higher normal intakes of total fat, SFA, cholesterol, total protein and animal protein, with total carbohydrate and dietary fibre intakes in the lower normal range. Other studies on urban black men in the Free State (Silvis & Mollentze, 1995; Slabber *et al*., 1997) and the Cape Peninsula Study (Bourne *et al*., 1993) also demonstrated a trend towards the adoption of a Westernised diet. These studies support the findings of the present study that the urbanised black men of the SANDF in Bloemfontein also

reflect a traditional phase towards westernised diets. The mean intakes for the micronutrients, however, met the RDA component of the DRIs, and indicate adequate micronutrient intakes, except for calcium and magnesium intake. In contrast with the present study, Bourne *et al*. (1993) found intakes below 67 percent of the RDAs for various vitamins and minerals, reflecting a nutritionally depleted diet. The adequate vitamin and mineral intake of the subjects could be ascribed to the fruits and vegetables available daily in the mess.

5.3 EFFECT OF SCFA SUPPLEMENT

The clinical effects of SCFA administration may be influenced by the method of administration employed (oral, rectal or intravenous). In this study, capsules coated with a thin layer of chelac for maximum absorption of SCFAs in the colon were administered orally. This prevented the acidity of the stomach from dissolving the capsules and enzymes in the small gut from digesting the capsules. The same method of administration was also used by Veldman *et al*. (1999) who also found that maximum absorption of acetate took place within the colon. Propionate and butyrate are almost completely metabolised by the liver and do not reach the systemic circulation. In addition, acetate is the only SCFA that appears in blood to any significant extent (Cummings *et al*., 1987a). Therefore, only acetate concentrations were measured to determine the absorption of the SCFA supplement. In the present study, SCFA supplementation caused significant increase in fasting acetate levels in the experimental group, and the rise in acetate levels seems large enough to have clinical effects (as mirrored in the changes in the haemostatic variables). The rise in acetate levels after SCFA supplementation in the present study corresponds with the rise in acetate levels after three weeks' supplementation of oat bran as shown by Bridges *et al*. (1992).

A significant decrease in glucose levels was found within both groups. Although other authors (Anderson & Bridges, 1984; Jenkins *et al*., 1990) found that SCFA might influence glucose levels, the decrease in glucose levels in the present study might be due to external factors other than supplementation as it occurred in both groups.

According to Table 5.1 the clinical effects of the SCFA supplement in the present study are compared with the findings of other human studies where acetate, propionate or butyrate was given. The effect of the SCFA supplement on the lipid profile and haemostatic risk factors in the present study resembled results of other studies in which the same variables were measured.

Table 5.1 Clinical effects of SCFAs supplementation

	TC	LDL	HDL/ %HDL	TG	Fibrin-ogen	FM	FNA	FVII	FVIII	References
SCFA	↕	↕	↑	↕	↕	↓	↑*	↓	↓	Present study
Acetate	↓	↕	↑	↓	↓**		↑*			Veldman *et al*. 1999
Acetate	↑									Wolever *et al*. 1989
Acetate + Propionate	↑			↑						Wolever *et al*. 1989
Acetate + Propionate	↕			↕						Wolever *et al*. 1991
Propionate	↕			↕						Wolever *et al*. 1989
Propionate	Slightly ↓			Slightly ↑						Todesco, 1991
Propionate	↕		↑	↑						Venter *et al*. 1990
Propionate	↕									Titius Ahearn, 1992

* Beneficial effects on fibrin network architecture
** Small, non-significant decrease
TC: total cholesterol
LDL: LDL cholesterol
HDL: HDL cholesterol
TG: triglycerides
FM: fibrin monomers
↕: unchanged
↓: decreased
↑: increased
FNA: fibrin network architecture
FVII: factor VII
FVIII: factor VIII

5.3.1 Lipid profile

The combination of SCFA supplementation (acetate, propionate and butyrate) caused no significant changes in the total cholesterol, LDL cholesterol and triglyceride concentrations of the normo lipedaemic subjects in the present study. A significant increase in HDL cholesterol concentration was, however, demonstrated in the experimental group after SCFA supplementation, while a small but non-significant change in the % HDL concentration occurred. In contrast, the placebo group showed a small non-significant decrease in the % HDL cholesterol concentration. However, the non-significant increase in the % HDL concentration in

the experimental group renders the significant increase in HDL cholesterol in this group of no clinical significance.

In contrast with the findings of the present study, Veldman *et al*. (1999) found that acetate supplementation alone decreases total cholesterol and triglyceride concentrations in hypercholesterolaemic men (Table 5.1). According to Wolever *et al*. (1989), acetate could reduce serum cholesterol levels by reducing FFA concentration, but Wolever *et al*. (1995) concluded that propionate inhibits the utilisation of acetate for cholesterol synthesis in men. Furthermore, Topping (1991) demonstrated that dietary propionate is absorbed with a totally different time course to that arising from the large bowel, making it a most unlikely mediator of cholesterol reduction by NSP. On the other hand, oral propionate does not affect LDL cholesterol in humans (Ahrens, *et al*., 1986), and rectal propionate may raise it (Royall *et al*., 1990).

The significant increase in the HDL cholesterol concentrations and the increase in the % HDL cholesterol concentration after SCFA supplementation are in congruence with a study done by Veldman (1999) where acetate supplementation alone caused a significant increase in % HDL cholesterol (Table 5.1). Venter *et al*. (1990) also found an increase in HDL concentration after the administration of propionate for seven weeks, and Singh *et al*. (1992) found that soluble fibre could cause an increase in HDL cholesterol concentration. Therefore, the assumption can be made that the combination of the SCFAs as fermentation products of soluble oat fibre could possibly have mediated the effect of an increase in HDL concentrations in the experimental group in the present study.

The subjects in the present study were normolipidaemic, and it could therefore be possible that the SCFA supplement had no effect on total cholesterol or LDL cholesterol. It could be possible, however, from the evidence found in other studies, that a combination of SCFA has beneficial effects on hypercholesterolaemic subjects. This should be investigated further.

5.3.2 Haemostatic risk factors

SCFA supplementation had no effect on the fibrinogen concentrations in the experimental group, but significantly decreased fibrin monomers, network fibrin content, factor VII activity and factor VIII activity. The combination of SCFAs caused a significant increase in the compaction of the fibrin network as well as a tendency of the mass to length ratio of the fibrin fibres to increase. This observation indicates that SCFAs may possibly have an effect on haemostasis, and supports the findings of Veldman *et al*. (1999) where acetate supplementation alone may have influenced haemostasis. Vorster *et al*. (1997) also stated that the protective effects of diet can be mediated through the haemostatic system, and to understand this possible role, it is necessary to give an overview of the haemostatic system.

The term haemostasis means prevention of blood loss (Guyton, 1991). Disturbance of haemostatic balance may lead either to thrombosis (clot formation inside a blood vesel), atherosclerosis, or a bleeding tendency (reviewed by Tanaka & Sueshi, 1993). Blood coagulates or turns into a solidified mass when plasma fibrinogen concentrations are converted by thrombin to monomers which polymerise in a fibrin network (Vorster, *et al*., 1997a). However, the rate of activation of fibrinogen by thrombin will increase significantly with increasing fibrinogen concentration and this leads to a drastic change in fibrin gel structure (Blombäck, 1994). The correlation of fibrinogen with fibrin monomers and the network fibrin content at baseline in the group as a whole in the present study possibly indicates that fibrinogen concentrations are equal to the fibrin monomer concentrations as well as the network fibrin content. However, after SCFA supplementation this correlation was not observed in the experimental group, but fibrinogen still correlates with network fibrin content in the Placebo group. This significant correlation possibly indicates that SCFAs might possibly decrease the fibrin monomer concentrations and network fibrin content independently of fibrinogen concentrations, since no change was observed in the fibrinogen concentrations after SCFA supplementation. However, because of the small number of subjects in the groups this finding should be interpreted with care. In accordance with the present study, Veldman *et al*. (1999) also found a significant decrease in network fibrin content, with a non-significant decrease in fibrinogen concentration after supplementation of acetate alone.

Furthermore, Marckmann *et al.* (1993) showed that low-fat, high-fibre diets followed for eight months by healthy volunteers had no change on fibrinogen levels, but improved fibrinolytic potential.

5.3.2.1 Fibrinogen fibrin monomers and fibrin network architecture

Measurement of fibrinogen concentration alone will only give limited information. Niewenhuizen (1994) mentions that the term 'fibrinogen' covers a large, heterogeneous family closely related to molecules whose functional properties may differ. Furthermore, the structure of fibrin network is not influenced by fibrinogen concentrations alone, but rather by the complex interplay of fibrinogen with other molecules in its direct surround. Measurement of polymerisation of fibrin monomers, as reflected in fibrin network structure and properties (Nair & Dhall, 1991), will give information on plasma factors which affect clot formation and the thrombogenicity of the clot (Collet *et al*., 1993). Thus, the significant decrease in the fibrin monomer concentration after SCFA supplementation in this study supports the theory that less fibrinogen is converted to fibrin, which eventually is incorporated into the fibrin networks. These structures are believed to be less atherogenic (Blombäck *et al*., 1992). The decrease in the fibrin content of the networks accompanied by the decrease in the fibrin monomers partially explains some of the altered network characteristics possibly due to altered fibrinogen conversion. The changes in the fibrin monomer concentration correlate significantly with changes in the acetate concentrations of the SCFA supplemented group. Therefore, it could be speculated that the SCFA supplementation could have a direct effect on the fibrin monomer concentration, but this relationship has not been shown in other studies.

The blood clotting system is normally in a dynamic steady state in which fibrin networks are constantly being laid down and subsequently dissolved. Local activation of the fibrinolytic system is an important antithrombotic mechanism (Gandrille & Aiach, 1990). It is known that any given network comprises a major network of thicker fibres and a minor network of thinner fibres (Nair *et al*., 1986). According to Blombäck *et al.* (1992), these gel structures are determined by thrombin and fibrinogen concentrations and an increase in these factors will result in tighter, less porous networks with thinner fibres and higher density of nodes. These

structures are more rigid, since the flow of liquid through them is impaired (Blomäck *et al*., 1992).

The mass length ratio of the fibrin fibres is determined by rates of generation of fibrin monomer and that of its assembly into fibrin fibres (Okada & Blombäck, 1983), indicating thicker fibrin fibres (Hantgan *et al*., 1985). Therefore, an increase in the mass length ratio of fibrin fibres found in this study in the SCFA supplemented group could possibly result in thicker fibrin fibres. The resulting increased thickness of fibres decreases the total contour length of the fibre thus increasing the permeability of the networks (Gabriel *et al*., 1992). Networks with higher compactions denote a decrease in the tensile strength of fibrin fibres and are therefore more collapsible (Nair *et al*., 1997). Moreover, lysability of the network is also related to the degree of cross-linking (Gaffney & Whitaker, 1979). Thus, the increase in compactions in the SCFA supplemented group indicates a decrease in the tensile strength of fibrin and a smaller degree of cross-linking. Networks with fibres of increased thickness and permeability are less resistant to lysis (Gabriel *et al*., 1992). In this study, it was shown that the SCFA supplement caused an increase in the lysis rate of the fibrin network. This is an important finding, as it may contribute towards a healthier haemostatic profile of the subjects.

The risk profile for CHD is most likely to be altered through SCFA supplementation by changing the fibrin network architecture independently of fibrinogen. Fatah *et* al. (1992) furthermore indicated that the formation of fibrin with thin fibres at an early age is related to CHD. Blombäck *et al*. (1992) also indicated that a grossly abnormal gel structure rather than simply the thightness and rigidity of the architecture is associated with progression of the atherosclerotic process.

5.2.3.2 Factor VII and Factor VIII

The SCFA supplement caused a significant decrease in factor VII activity. The decrease in factor VII activity in the supplementation group indicated a possible direct effect of SCFAs (the fermentation products of oat bran) on factor VII activity. In support of the findings of the present study, Marckman and Jespersen (1996) found a decrease between 5 to 25 percent in factor VII activity after oat bran

supplementation. Marckman *et al*. (1994) also indicated a decrease in factor VII levels on a low-fat, high-fibre diet, and Haines *et al*. (1980) showed that factor VII was lower in vegetarians. In accordance with Marckmann *et al*. (1994) and Haines *et al*. (1980), Tracy *et al*. (1995) also found that dietary modification might have beneficial effects on factor VII activity as a coronary risk factor. Furthermore Hoffman *et al*. (1992) indicated a correlation between cholesterol levels and factor VII activity, and the NCEP also indicated that an increase in soluble fibre will cause a decrease in total serum cholesterol levels (ATPII, 1994). Therefore, it could be possible that factor VII activity is lowered through the cholesterol-lowering effect of soluble dietary fibre, but the present study indicates a possibility that the positive effect of dietary intake, especially high-fibre, on factor VII activity could directly be mediated through SCFAs.

Factor VII forms part of the cascade eventually ending in the activation of prothrombin to thrombin and thus, the conversion of fibrinogen to fibrin (Swales & de Bono, 1993, p. 143). Factor VII activity has been found to correlate positively with network fibrin content. Thus, a decrease in factor VII activity could possibly decrease the conversion of fibrinogen to fibrin. Factor VII activity inversely correlated with factor VIII activity. No correlation between factor VII and factor VIII activities could be found in other studies. Low factor VII activity is also associated with a decrease in the tensile strength of fibrin fibres as indicated in the inverse correlation between factor VII activity and compactions in the Placebo group.

In the present study, SCFA supplementation also caused a decrease in factor VIII activity. Simpson *et al*. (1982) also indicate a decrease in factor VIII after increased dietary fibre intake. As indicated by Pan *et al*. (1997) and Tracy *et al*. (1995), factor VIII is positively associated with atherosclerosis and is also independently associated with abnormal wall motion in CVD. Factor VIII circulates in the plasma where it binds to arteries that have lost their endothelial cell lining (Hensyl, 1990, p. 560). Although no significant decrease in factor VIII levels through dietary modification have been reported in the past, it is possible that an increase in soluble dietary fibre might cause a significant decrease in factor VIII activity. Factor VIII activity has also been found to inversely correlate with factor VII activity.

5.4 SUMMARY

The study group used in the present study was homogeneous as far as all the measured variables are concerned. The usual diet of the subjects shows a tendency towards the adoption of a high-fat, low-carbohydrate, low-fibre atherogenic westernised diet. Although favourable lipid profiles were evident in the present study group, the higher normal total cholesterol levels further support the trend towards Westernisation.

The rise in fasting acetate concentration found in this study after supplementation possibly indicates that the SCFA supplement had a direct effect on some of the variables measured in the present study.

The combination of SCFAs possibly caused, although not clinically significant, a statistical significant increase in the HDL cholesterol concentrations of the subjects.

SCFA supplementation significantly decreased fibrin monomer concentrations and the network fibrin content, while no change in fibrinogen levels was observed. This effect of the specific combination of SCFAs (similar to the fermentation products of oat bran) on fibrinogen, fibrin monomer concentrations and network fibrin content has not been investigated in other studies yet. The finding that fibrin monomer concentrations and network fibrin content decrease independently of fibrinogen concentrations after SCFA supplementation possibly indicates that fibrinogen need not be present to beneficially change the haemostatic risk profile. It further indicates that SCFA might possibly play an essential role in the alteration of hypercoagulable states. In support of this finding, the fibrin network was also less atherogenic after SCFA supplementation. This can be seen in the thicker fibres, which increased the permeability of the fibres, as well as the decrease in tensile strength leading to more collapsible fibres. Furthermore, factor VII and factor VIII activity, which is also involved in the haemostatic system, also decreased after SCFA supplementation. The lowering effect of the SCFA supplement on the factor VII activity in the present study corresponds with the findings in other studies where high-fibre and specially oat bran supplementation have a lowering effect on factor VII levels. The present study was, however, the first to indicate a lowering effect of SCFAs on factor VIII

activity. It could therefore be speculated that SCFA might beneficially influence factor VII and factor VIII activity.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 INTRODUCTION

The adoption of a westernised lifestyle among black populations will increase the prevalence of degenerative western diseases, such as CHD and stroke (Hubbard *et al*., 1994). The prevalence of stroke is especially high in westernised black populations (Joubert, 1991). The characteristics of a westernised lifestyle often include increased socio-economic status, increased prevalence of coronary risk factors (including raised fibrinogen concentrations) and the adoption of high-fat, low-fibre diets (Venter *et al*., 1992; Bourne *et al*., 1993; Mollentze *et al*., 1995). The westernised diet can be seen as one of the controllable risk factors for the development of atherogenic and thrombotic diseases such as CHD and stroke (Ulbricht & Southgate, 1991). Vorster *et al*. (1997) indicated that diet could possibly influence haemostasis. This motivation supports the need for the evaluation of dietary modifications that could beneficially influence risk factors associated with Westernisation.

The aim of the study was to determine the effect of a combination of SCFA supplement (acetate:propionate:butyrate - 65:19:16) on plasma fibrinogen levels, some other haemostatic risk factors including fibrin network architecture, factor VII and factor VIII, as well as some coronary risk factors in westernised black men. Each subject received 12 capsules daily (4 with each meal) containing the total amount of 6g SCFAs per day. This amount was equal to the amount of SCFAs produced by an intake of 15g oat bran fibre. The standardised techniques used for the execution of the study, as described in chapter 3, assured that the results of the study can be seen as valid and reliable. A placebo group was included in the study to measure the effect of the supplement.

The study group was of a homogeneous nature, and the subjects were eating all their meals at the same mess. This study group can therefore be seen as the suitable group to serve the purpose of the study. Although the subjects were normolipidaemic, total cholesterol levels, factor VII and factor VIII activity were within the higher normal ranges. Further, the usual dietary pattern derived from the 24-hour recall indicated a tendency towards a Westernised diet among the subjects. SCFA supplementation was sustained for five weeks and was very well tolerated by the subjects with no sign of side effects. The usual dietary pattern of the subjects did not change much and was nearly similar at the end of the study as at baseline. It is therefore suspected that the beneficial effects seen in the study could be ascribed to SCFA supplementation. The following conclusions can be made from the findings of the present study according to the pre-set goals.

6.2 CONCLUSIONS

The study showed that the SCFA supplement had no effect on the anthropometry, total cholesterol, LDL cholesterol or triglyceride levels of normolipidaemic subjects. The supplement caused a statistical significant increase in only the HDL cholesterol concentrations of the experimental group although of no clinical significance. Although this specific combination of SCFAs had a significant effect only on the HDL cholesterol in this study, a decrease in total cholesterol was found from acetate (Veldman *et al*., 1999) and propionate alone and (Todesco, 1991) in subjects with hypercholesterolaemia. It can be concluded that this specific combination of SCFAs might possibly influence the lipid profile of dyslipidaemic subjects. However, due to the small sample size the results on the lipid profile should be interpreted with care and should be investigated further on larger sample sizes.

Lowering of the concentration of the fibrinogen molecule could possibly be associated with a decreased risk for the development of CHD and stroke. It is also believed that the dimensional packing of fibrin, which is formed after the enzymatic breakdown of the fibrinogen molecule, may be used as an indicator of the risk associated with fibrinogen concentrations. Previous studies showed that fibrin networks might be altered without a change in the fibrinogen concentrations

(Veldman *et al*., 1999). These results were supported by the findings of the present study. This finding is very important as it may indicate that the risk of CHD and stroke associated with fibrinogen concentrations may be decreased without a change in the fibrinogen concentrations, but rather mediated through the formation of less atherogenic network structures. The SCFA supplementation in the present study caused formation of softer, more porous networks with thick fibres and fewer nodes. These structures are less resistant to lysis and are also more deformable and plastic, since fluid easily escapes from the structures (Blombäck *et al*., 1992), and is therefore thought to be less atherogenic. The structure of fibrin network is not influenced by fibrinogen concentrations alone, but rather by the complex interplay of fibrinogen with other molecules in its direct surround. Therefore, it can be concluded that changes in plasma fibrinogen levels possibly need not be present to reduce cardiovascular risk.

The most important finding of this study was the significantly positive alteration of the fibrin network architecture, accompanied by a decrease in the formation of fibrin due to a decrease in the fibrin monomer concentrations. The fibrin monomers are derived from fibrinogen, after the enzymatic breakdown of the fibrinogen molecule. Raised levels of fibrin monomer concentrations are associated with a hypercoagulable state. These monomers associate with each other to form an insoluble fibrin network. No change in fibrinogen concentrations was observed within the subject group. However, the SCFA supplement caused a significant decrease in fibrin monomer concentrations, as well as network fibrin content. This directly indicates that the activity of the fibrinogen molecule was altered, as less fibrinogen was converted to fibrin monomers, and less monomers converted to the amount of fibrin incorporated into the fibrin networks. It could be speculated that the fibrinogen concentration stays constant due to the fact that less fibrinogen is converted to monomers, thus counteracting a decrease in circulating plasma fibrinogen. However, this should be investigated further.

SCFA supplementation significantly decreased factor VII and factor VIII activity, and it could therefore be speculated that SCFA supplementation had a direct effect on decreasing factor VII and factor VIII activity. Fibrinogen concentrations as well as

factor VII and factor VIII activities are associated with stroke (Cushman *et al*., 1996), and a high incidence of stroke has been reported among black populations (Kahn & Tollman, 1998). According to these findings, it is possible that increased factor VII and factor VIII activity could be treated or possibly prevented by an increased intake of SCFAs. This finding, however, needs further investigation. It could further be concluded that factor VII and factor VIII activity should be included when assessing the risk of CHD and stroke in black populations.

The rise in fasting acetate levels in the SCFA supplemented group seemed large enough to expain the clinical effects observed in this study. There is enough evidence to conclude that some of the protective effects of diet against the development of atherosclerosis, thrombosis and resultant CHD are mediated in part through the effect of SCFAs on haemostasis.

6.3 RECOMMENDATIONS

The findings of the present study indicate that further research is needed to evaluate the effect of this combination of SCFAs that could be derived from the fermentation products of oat bran on dyslipidaemic subjects as well as haemostasis, and the risk factors associated with atherosclerosis and thrombosis.

The effects of acetate, propionate and butyrate alone were intensively researched, and indicated effects on carbohydrate metabolism (Wolever, 1995), lipid metabolism (Anderson, 1995), and haemostasis (Veldman *et al.*, 1999). Several authors also indicated beneficial effects of dietary fibre on the lipid profile (Glore *et al*., 1994) and haemostasis (Vorster *et al*., 1997). Further research is therefore needed on different combinations of SCFAs as fermentation products from different fibres, on these variables.

The 12 capsules of SCFAs administered in the present study, which are equal to the fermentation products of 15g of fibre from oat bran indicated beneficial effects on haemostasis. Although the subjects co-operated well in consuming this amount of capsules, it could be impractical to do so over a long period. The amount of SCFAs

needed to indicate a clinical effect on coronary and haemostatic risk factors should therefore be investigated further.

People should be educated to include more soluble fibre products, especially oats in their diet. Glore *et al.* (1994) indicated that 25-100g of oat bran is needed to produce a lipid-lowering effect. 100g uncooked oat bran contains 20g of soluble dietary fibre. The 12 capsules consumed daily in the present study consisted a total amount of 6g of SCFAs (acetate:propionate:butyrate – 65:19:16), which are equal to the fermentation products from 15g oat bran fibre. Therefore, 6g of this specific combination of SCFAs is equal to 75g uncooked oat bran or two cups cooked oat bran. The effect of oat bran, compared to the effect of SCFAs on coronary and haemostatic risk factors, should therefore be investigated to indicate if the results obtained will be similar. These findings could also indicate the effectiveness of the development of a SCFA supplement as a food supplement.

Furthermore, the effect of dietary factors on haemostatic risk factors, including the fibrin network structure, fibrin monomer concentrations, factor VII levels and factor VIII levels should be further investigated.

It is also recommended that fibrin network structure and fibrin monomer concentration should further be investigated as risk factors for CHD and cerebrovascular disease.

The study clearly underlines the need for further investigation. Another study using more subjects would be ideal, however, it will be difficult to perform because of the practical implications.

The value of this study lies in the finding that SCFAs (oat bran) beneficially influenced the fibrin monomer concentrations and fibrin network structure independent of fibrinogen concentrations. This indicates that fibrinogen concentrations as well as fibrin monomer concentrations and network fibrin concentrations should possibly be considered as risk factors for cerebrovascular disease and coronary heart disease. The results of this study can be used as basis

for further exciting research on SCFAs as mediator for the effect of soluble dietary fibre on several health parameters, as well as for the role of diet in haemostasis.

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OPSOMMING

Die voorkoms van die westerse siektes arteriosklerose, koronêre hartsiektes (KHS) en beroerte is besig om vinnig wêreldwyd en in Suid-Afrika toe te neem. Serebrovaskulêre siektes (beroerte) is ‘n belangrike oorsaak van sterftes in swart populasiess in Suid-Afrika. KHS en beroerte neem selfs verder toe wanneer die risikofaktore verhoog as gevolg van verwestering wat ‘n verandering in leefstyl en dieet teweeg bring. Verhoogde fibrinogeenvlakke, wat meer onder verwesterde swarte as blanke mans voorkom, word as ‘n belangrike onafhanklike risikofaktor vir beroerte beskou. Die dieet speel verder ook ‘n belangrike rol in die ontwikkeling van arteriosklerose en trombose en word moontlik gedeeltelik deur hemeostase gemedieer. Die omsigtige lae-vet, hoë-vesel dieet kan moontlik hemeostase gunstig beïnvloed. Hawersemels (oplosbare vesel) is meer spesifiek aangedui as ‘n belangrike dieetfaktor in sommige voordelige effekte van dieet op koronêre risikofaktore en hemeostase. Die metaboliese effek van dieetvesel word geassossieer met kortketting vetsure (KKV) wat in die kolon gevorm word as gevolg van fermentasie. Volgens beskikbare literatuur kan KKV moontlik ‘n voordelige effek op die koronêre en hemeostatiese risikofaktore hê. Min bewyse bestaan egter oor die effek van ‘n kombinasie van KKV op fibrinogeenkonsentrasies en ander hemeostatiese risikofaktore.

Die hoofdoelwit van die studie was om die effek van ‘n kombinasie van KKV wat die fermentasieprodukte van hawersemels verteenwoordig (asetaat:propionaat:butiraat) op plasmafibrinogeenkonsentrasies en verwante risikofaktore vir KHS en beroerte in verwesterde swart mans te bepaal.

Die studie was ‘n ewekansige, dubbelblinde kliniese proef. 22 proefpersone, geselekteer volgens spesifieke insluitingskriteria en hoog normale fibrinogeenvlakke, is in die studie ingesluit. Die proefpersone is ewekansig in ‘n eksperimentele groep (n=11) en ‘n kontrole groep (n = 10) verdeel. Supplementasie van 12 kapsules daagliks het vir vyf weke plaasgevind. Plasmafibrinogeenkonsentrasies, fibrienmonomeerkonsentrasies, fibriennetwerkeienskappe, faktor VII en faktor VIII aktiwiteit, serumlipiede, vastende glukose vlakke, volbloedtelling, en vastende asetaatkonsentrasies is aan die begin en die einde van die studie gemeet. Die

respondente se gebruiklike voedselinname is met ‘n 24-uur herroep en voedselfrekwensie vraelys bepaal.

Volgens die basislyn resultate was die steekproef ‘n kliniese en fisiologiese gesonde homogene groep. Beide groepe was normolipidemies met ‘n neigings tot hoog normale totale cholesterolvlakke, faktor VII en faktor VIII aktiwiteit. Volgens die 24-uur herroep metode het die respondente ook ‘n neiging tot die aankweek van westerse eetpatrone getoon. KKV supplementasie het geen effek op fibrinogeenkonsentrasies gehad nie, maar het die fibrienmonomeerkonsentrasies en die fibrien netwerk inhoud, faktor VII en faktor VIII betekenisvol verlaag. Verder is ‘n betekenisvolle verhoging in die kompaksies van die fibriennetwerke gevind asook ‘n neiging tot verhoging in die massa/lengte verhouding van die fibrienvesels. Hoewel, KKV supplementasie die HDL cholesterolkonsentrasiess statisties betekenisvol verander het na vyf weke, wass die effek nie klinies betekenisvol nie.

Die bevindings van die studie dui aan dat KKV supplementasie moontlik ‘n direkte effek op hemeostase kan hê, spesifiek ten opsigte van die fibriennetwerkstrukture, faktor VII en faktor VIII asook fibrienmonomeerkonsentrasies. Hierdie bevindings dui moontlik aan dat KKV ssupplementasie ‘n sterk beskermende eienskap teen arteriosklerose en trombose teweeg kan bring.

Die gevolgtrekking kan gemaak word dat die voordelige effek van oplosbare vesel moontlik bemiddel word deur die produksie van KKV. Dit was duidelik dat, alhoewel die fibrinogeenkonsentrasies na supplementasie onveranderd gebly het, die KKV wel die fibriennetwerkstrukture positief verander het om minder aterogenies te wees. Die studie dui dus ook aan dat die beskermende effek van dieetvesel op KHS moontlik deur die effek van KKV op die fibriennetwerke bemiddel word. Hieruit volg die aanbeveling dat die rol van fibriennetwerke as risikofaktor vir hartsiektes, asook die effek van die dieet op hierdie netwerke verder bestudeer moet word.

Sleutelwoorde: Verwestering, dieetvesel, KKV, fibrinogeen, fibriennetwerkstrukture, faktor VII en faktor VIII.

SUMMARY

The incidence of the western diseases, atherosclerosis, CHD and stroke is progressively rising in black populations worldwide and in South Africa. Stroke is an important cause of death in black populations in South Africa and may increases even further if risk factor (coronary and some haemostatic risk factors) prevalence is altered by change in lifestyle and diet, westernisation and migration to an urban environment. Raised fibrinogen levels which are more prevalent in westernised black men than white men, are accepted as an important risk factor for stroke and CHD. It is believed that the possible protective effects of diet against the development of atherosclerosis and thrombosis could be mediated, in part, through haemostasis. A prudent low-fat, high-fibre diet may favourably influence haemostasis. More specifically, oat bran (soluble fibre) has been shown to have beneficial effects on some coronary risk factors and haemostasis. The physiological effects of dietary fibre are strongly related to SCFAs, which are produced by colonic fibre fermentation. According to available literature, SCFAs could possibly have a beneficial effect on lipid profiles and haemostatic risk factors. Little information is, however, available on the effect of a specific combination of SCFAs on fibrinogen levels and other haemostatic factors in human subjects.

The main objective of the study was to examine the effect of a combination of SCFAs, resembling oat bran (acetate:propionate:butyrate – 65:19:16) on plasma fibrinogen levels, some haemostatic risk factors and other related risk factors for CHD and stroke in westernised black men.

The study was a randomised, placebo-controlled, double-blind clinical trial. 22 subjects falling within a pre-determined set of inclusion criteria, and with higher normal fibrinogen levels were randomly selected into an experimental group (n = 11) and placebo group (n = 10). Supplementation of 12 capsules daily was sustained for five weeks. Total plasma fibrinogen, fibrin monomer concentration, fibrin network properties, factor VII and factor VIII activity, serum lipids, glucose concentrations, some metabolic indicators and fasting acetate concentrations were measured at baseline and at the end of supplementation, in all subjects. The usual dietary intake

of the subjects was obtained using a food frequency questionnaire and a 24-hour recall.

According to the baseline results, the subject group was homogeneous with an apparently healthy clinical and physical appearance. Although both subject groups had a favourable coronary and haemostatic risk profile, total cholesterol levels as well as factor VII and factor VIII activity were in the higher normal ranges. Furthermore, the 24-hour recall indicated a tendency towards the adoption of an atherogenic Westernised diet. Although SCFA supplementation had no effect on the fibrinogen concentrations, a significant decrease was observed in the fibrin monomer concentrations, network fibrin content, factor VII and factor VIII activity. A significant increase was observed in the compaction of the fibrin networks, as well as a tendency for the mass to length ratio of the fibrin fibres to increase. Furthermore, a statistically significant although not clinically significant increase was indicated in HDL cholesterol concentrations after SCFA supplementation.

It was evident from these findings that SCFA supplementation may have a direct effect on haemostasis, especially the fibrin network characteristics, factor VII and factor VIII activities, as well as fibrin monomer concentration. This observation suggests that SCFA supplementation may have a strong protective effect against atherosclerosis and thrombosis.

In conclusion, the hypothesis that soluble dietary fibre will influence fibrinogen concentrations and other haemostatic risk factors through production of SCFAs, was proven to be partially true. It was clear that, although fibrinogen concentration was not influenced by SCFA supplementation, beneficial effects on the fibrin network architecture and the positive cascade effect on haemostasis may be a direct effect of SCFAs supplementation. The study further indicated that the known protective effects of dietary fibre on CHD could partially be mediated through effects of SCFAs on fibrin networks. It is recommended that the role of fibrin networks as a risk factor for CHD and the effect of diet on haemostasis should be further investigated.

Keywords: Westernisation, dietary fibre, SCFAs, fibrinogen, fibrin network architecture, factor VII, factor VIII.