THE EFFECT OF SHORT-CHAIN FATTY ACIDS ON SOME

HAEMOSTATIC RISK MARKERS IN WESTERNISED BLACK MEN

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BLOEMFONTEIN

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DECLARATION OF INDEPENDENT WORK

I, LEBOGANG FRANCIS MOGONGOA, hereby declare that this research project submitted to the Central University of Technology, Free State for the degree MAGISTER TECHNOLOGIAE: BIOMEDICAL TECHNOLOGY is my own independent work that has not been submitted before to any institution by me or any other person in fulfilment of the requirements for the attainment of any qualification.

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VERKLARING VAN ONAFHANKLIKE WERK

Ek, LEBOGANG FRANCIS MOGONGOA, verklaar hiermee dat hierdie navorsingsprojek wat ingehandig word aan die Sentrale Universiteit vir Tegnologie, Vrystaat, vir die graad MAGISTER TECHNOLOGIAE: BIOMEDIESE TEGNOLOGIE, my eie werk is wat nie deur myself of enige ander persoon aan enige instansie, vir die verwerwing van enige kwalifikasie, voorgelê is nie.

_____   _____

HANDTEKENING VAN STUDENT   **DATUM**

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SUMMARY

Cerebrovascular disease and coronary heart disease (CHD) are of the most important causes of morbidity and mortality amongst South Africans. The risk factor prevalence for stroke and CHD becomes altered by changes in lifestyle, including diet. In general it is suggested that lifestyle management should be the first choice when having to treat patients with increased cardiovascular risk.

The prudent low-fat, high-fibre diet is regarded as an apparently healthy diet. It is suspected that this diet is effective for the control of known coronary risk factors as well as raised clotting factors. Research studies have shown the addition of dietary fibre to the diet as a promising therapeutic agent for the limited control of known coronary risk factors. The physiological effects of dietary fibre in humans are significantly influenced by the degree to which fibre is fermented in the colon. Fermentation results in the production of short-chain fatty acids (SCFAs); acetate, propionate and butyrate.

The aim of this study was to examine the possible effects of different combinations of short-chain fatty acids on some metabolic risk markers. In this study a group of westernised African male volunteers was recruited and randomly assigned to three groups. Group one received a placebo. Group two received a supplement containing 50% acetate and 50% propionate. Group three received a SCFA supplement in the ratio of 70% acetate, 15% propionate and 15% butyrate. Supplementation was sustained for a period of six weeks. Blood samples were drawn during the different visits.

At baseline the study group represented a group of black African men without any apparent metabolic or physical abnormalities. All measured variables fell within the normal range. In the placebo group, there was a statistically significant decrease in plasma fibrinogen levels from baseline to the end of supplementation. In the acetate-propionate supplement study group a statistically significant decrease in factor VIII (from 91.1 ± 11.2 to $90.9 \pm 8.3\%$, respectively), and ATIII (from 114.3 ± 13.1 to $108.34 \pm 9.5\%$), as well as a statistically significant decrease in low-density lipoprotein cholesterol (LDL-C) from 3.10 ± 0.79 to 2.64 ± 0.73 mmol/L. The significant increase in %HDL-C from 26.3 ± 6.5 to $30.2 \pm 9.3\%$ should also be noted. Both triglycerides (8%) and plasma fibrinogen (2%) showed a statistically significant increase. However, these changes are of no clinical significance.

For the high-acetate supplement study group (with the addition of butyrate), a statistically significant decrease in factor VII (from 102.5 ± 13.7 to $101.1 \pm 6.4\%$), VIII (from 92.6 ± 12.8 to $87.6 \pm 6.0\%$), ATIII (from 109.2 ± 16.0 to $103.0 \pm 9.9\%$) as well as fibrin monomer concentration (from 13.9 ± 2.2 to 12.1 ± 3.6 mg/L), were measured. Fibrin network compaction increased significantly from 14.2 ± 4.6 to $13.7 \pm 4.0\%$. Other changes include a statistically significant increase in the serum-TC of 4.2%.

From the results it is evident that the acetate-propionate supplement, with exclusion of butyrate, has a beneficial effect on metabolic parameters when compared to a high-acetate-propionate supplement. The results do provide evidence of a possible therapeutic application for the propionate-acetate containing supplement. The specific mechanism should, however, still be investigated.

It can be concluded from this study that acetate, propionate and butyrate each have different effects on human metabolism. It is evident that the use of a mixture of acetate and propionate may have a beneficial effect on patients at risk of developing CVD. Further studies that investigate the optimum ratio of these two products may lead to the development of a naturally derived therapeutic product for the prevention or treatment of CVD in black African men, as well as the population at large.

OPSOMMING

Serebrovaskulêre siekte en koronêre hartsiekte is van die belangrikste oorsake van morbiteit en mortaliteit by Suid-Afrikaners. Die prevalensie van risikofaktore vir beroerte en koronêre hartsiektes word deur veranderinge in lewensstyl, insluitende dieët bepaal. Oor die algemeen word dit aanbeveel dat die manipulasie van lewenstyl die eerste keuse vir behandeling van pasiënte met verhoogde kardiovaskulêre risiko moet wees.

'n Dieët met lae vet- en hoë veselinhoud word as 'n gesonde dieët beskou. Daar word vermoed dat hierdie dieët effektief is vir die kontrolering van bekende koronêre risikofaktore sowel as verhoogde stollingsfaktore. Navorsingstudies het getoon dat die byvoeging van dieëtvesel by die dieët 'n belowende terapeutiese agens is vir die beperkte kontrolering van bekende koronêre risikofaktore. Die fisiologiese effekte van veselinname in mense word beïnvloed deur die mate wat vesel in die kolon gefermenteer word. Fermentasie lei tot die produksie van kortkettingvetsure; asetaat, propionaat en butiraat.

Die doel van die studie was om die moontlike effek van verskillende kombinasies van kortkettingvetsure op metaboliese risikomerkers te toets. In hierdie studie is 'n groep vrywillige verwesterde swart mans gevra om deel te neem en ingedeel in drie groepe deur randomisering. Groep een het 'n plasebo ontvang. Groep twee het 'n aanvulling ontvang wat 50% asetaat en 50% propionaat bevat. Groep drie het 'n kortkettingvetsuuraanvulling ontvang in die verhouding van 70% asetaat, 15% propionaat en 15% butiraat. Aanvullings is vir 'n periode van ses weke toegedien. Bloedmonsters is tydens die verskillende besoeke versamel.

Die studiegroep het aanvanklik uit 'n groep swart mans sonder enige metaboliese of fisiese abnormaliteite bestaan. Alle gemete veranderlikes het binne die normale waardes geval. In die plasebogroep was daar 'n statistiese betekenisvolle verlaging in plasma-fibrinogeenvlakke vanaf basislyn tot met die einde van die aanvullings. Die asetaat-propionaataanvullingstudiegroep, het 'n statistiese betekenisvolle verlaging in faktor VIII (van 91.1 ± 11.2 tot 90.9 ± 8.3%, respektiewelik) en ATIII (van 114.3 ± 13.1 tot 108 ± 9.5%) getoon, sowel as 'n statistiese betekenisvolle verlaging in LDL-C van 3.10 ± 0.79 tot 2.64 ± 0.73 mmol/L. Die betekenisvolle verhoging van %HDL-C van 26.3 ± 6.5 to 30.2 ± 9.3% moet ook in aanmerking geneem word. Beide trigliseriede (8%) en plasma-fibrinogeen (2%) het 'n statistiese betekenisvolle verhoging getoon. Hierdie veranderinge is egter van geen kliniese waarde nie.

Die hoë-asetaatstudiegroep (met die byvoeging van butiraat), het 'n statistiese betekenisvolle verlaging in faktor VII (van 102.5 ± 13.7 tot 101.1 ± 6.4%), VIII (van 92.6 ± 12.8 tot 87.6 ± 6.0%), ATIII (van 109.2 ± 16.0 tot 103.0 ± 9%) getoon, sowel as fibrienmonomeerkonsentrasie (van 13.9 ± 2.2 tot 12.1 ± 3.6mg/L). Fibriennetwerkkompaksie het betekenisvol verhoog vanaf 14.2 ± 4.6 tot 13.7 ± 4.0%. Ander veranderinge sluit in 'n statistiese betekenisvolle verhoging in totale serum-cholesterol van 4.2%.

Volgens die resultate is dit beduidend dat die asetaat-propionaataanvulling, met uitsluiting van butiraat, 'n voordelige effek op metaboliese parameters het wanneer dit vergelyk word met 'n hoë-asetaat-propionaataanvulling. Die resultate voorsien bewyse van 'n moontlike terapeutiese effek deur die propionaat-asetaat bevattende aanvulling. Die spesifieke meganisme moet nog ondersoek word.

Die studie toon dus dat asetaat, propionaat en butiraat elk verskillende effekte op menslike metabolisme het. Daar is bewys dat die gebruik van 'n mengsel van asetaat en propionaat 'n voordelige effek het op pasiënte met 'n risiko het om serebrovaskulêre siekte te ontwikkel. Verdere studies wat die optimale verhouding van hierdie twee produkte ondersoek, kan lei tot die ontwikkeling van 'n natuurlike terapeutiese produk vir die behandeling van serebrovaskulêre siekte in swart mans, sowel as die bevolking as geheel.

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LIST OF ABBREVIATIONS

α	Alpha
β	Beta
γ	Gamma
μ	Micro
μT	Mass length ratio from turbidity
AIDS	Acquired Immunodeficiency Syndrome
ATIII	Antithrombin III
ADP	Adenosine diphoshate
CHD	Coronary heart disease
CoA	Co-enzyme A
CRP	C-reactive protein
CVD	Cerebrovascular disease
EDTA	Ethylenediaminetetraacetic acid
FBC	Full blood count
HCT	Haematocrit
HDL-C	High density lipoprotein cholesterol
HGB	Haemoglobin
HIV	Human immunodeficiency virus
LDL-C	Low density lipoprotein cholesterol
NaOH	Sodium hydroxide
NEFA	Non-esterified fatty acids
NFC	Network fibrin content
PAI-1	Plasminogen activator inhibitor-1

PLT	Platelets
RBC	Red blood cells
SCFA	Short-chain fatty acid
SCFAs	Short-chain fatty acids
TC	Total cholesterol
TG	Triglycerides
tPA	Tissue plasminogen activator
VLDL	Very low density lipoprotein
WBC	White blood cells

APPENDIXES

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Cerebrovascular disease and coronary heart disease (CHD) are 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; Qizilbash, 1995; Kahn & Tollman, 1999) as well as black population in the United States of America (Iso *et al.*, 1989). The risk factor prevalence for stroke and CHD becomes altered by changes in lifestyle and diet. Westernisation and migration to an urban environment causes an even further rise in the risk of stroke and CHD (Steyn *et al.*, 1991; Seedat *et al.*, 1992; Bourne *et al.*, 1993; Mollentze *et al.*, 1995; Solomons & Gross, 1995). 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)

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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.

Westernised African populations tend to have elevated fibrinogen concentrations (Venter *et al.*, 1992; Slabber *et al.*, 1997). Venter *et al.* (1992) also demonstrated that westernisation of Africans tends to raise fibrinogen concentrations even before an increase in serum lipoproteins is observed. Raised plasma fibrinogen concentration is 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 fibrin network structures, factor VII, factor VIII and C-reactive protein (CRP), are also accepted as important for the development of these westernised diseases (Kannel *et al.*, 1987; Yarnell *et al.*, 1991; Blombäck *et al.*, 1992, Heinrich *et al.*, 1994, Pan *et al.*, 1997 Mills *et al.*, 2002).

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 for the control of known coronary risk factors (hyperinsulinaemia, hyperlipidaemia, hypertension, and obesity) 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 & 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 improve not only glucose tolerance and reduce serum lipid and lipoprotein concentrations, but also reduce fibrinogen concentrations. The effect of diet on haemostatic risk factor is however, still controversial and not well established. This underlines the importance to investigate 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 (Bugaut & Bentejac, 1993; Muir *et al.*, 1995). Total short-chain fatty acid (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 decrease on fibrinogen

concentration, but found a significant difference in the characteristics of fibrin networks. The decrease in network fibrin content indicates that less fibrinogen is converted to fibrin, which is eventually incorporated into the fibrin networks, which are believed to be less atherogenic (Veldman *et al.*, 1999). From these limited observation it is evident that there is a possible association between dietary fibre of SCFAs, fibrin network architecture and some other haemostatic risk factors. This observation, however, lacks thorough investigation.

1.2   HYPOTHESIS

Hypothesis I

H0 - The increase in colonic propionate by means of supplementation will not significantly improve the haemostatic and lipid profiles.

H1 - The increase in the colonic propionate by means of SCFA salt supplements will improve the haemostatic and lipid profiles.

Hypothesis II

H0 - Doubling SCFA in the large intestine by supplementation of SCFA at an amount (molar) equivalent to SCFA generated by fermentation of 15 grams mixed fibres (with an increased acetate concentration) will not significantly improve indicators of the haemostatic and lipid profiles.

H1 - SCFA supplements in the large intestine will result in a significant improvement of haemostatic and lipid profiles; with significantly weaker and more permeable fibrin networks, coupled with improved lipid profiles. These metabolic changes would indicate a lower risk for cardiovascular disease.

1.3   OBJECTIVE

The objective of the study was to test the effects of different short-chain fatty acids ratio on some haemostatic and lipid risk markers for cardiovascular disease in westernised black men.

1.4   DISSERTATION STRUCTURE

The first chapter contain the introduction with the aim and motivation for the study. Chapter two deals with the link between the different variables examined in this study and contains information needed for the understanding and interpretation of the study. The materials and methods used during the study are presented in chapter three, while the findings are given in chapter four. Chapter five discusses the results, and six presents the meaning of the findings and recommendations, while seven contains the references used in this dissertation.

CHAPTER 2

LITERATURE REVIEW

2.1   INTRODUCTION

There are two separate but interrelated aspects involved in this study. These factors include risk markers for coronary heart disease and stroke, and lifestyle (diet, exercise, stress, etc.). Coronary heart disease and stroke are considered as degenerative western diseases which in most cases can be prevented by a change in lifestyle, for example diet, physical activity and psychological well being. Coronary heart disease and stroke in turn involves haemostatic and lipid risk markers. Africans have the lowest incidence of these westernised disorders, in comparison to other South African population groups. This is thought to be due to the prudent, low-fat high fiber diet which Africans consume. However with increased urbanization and adoption of a western, high-fat low-fiber diet, it seems set that this will cause an increase in the incidence of the westernized diseases among the African population. The protection seems to center around the intake of dietary fiber, which is fermented in the large intestine to short-chain fatty acids. Another factor that seems set to increase the incidence of CHD and stroke is the lipodystrophy associated with human immunodeficiency virus (HIV) antiretroviral treatment. This chapter will be

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used to elaborate the link amongst the production of short-chain fatty acids and the development of lifestyle associated diseases and lay the foundation for the understanding of the results and the rest of this dissertation.

2.2   DEFINITION OF TERMS

2.2.1 Coronary Heart Disease

Coronary heart disease (CHD) is in most instances due to obstruction of coronary vessels by atherosclerosis or thrombosis, singly or in combination (Ulbright & Southgate, 1991) that may result in myocardial infarction, sudden death, and angina pectoris. Atherogenesis and arterial thrombus are closely linked and each contributes to the other, as indicated by both autopsy and coronary arteriography studies. There is a dynamic interaction between spasm, thrombus and atherosclerotic plaque preceding acute coronary occlusion (Packham & Kinlough-Rathbone, 1994).

2.2.2 Stroke

Stroke refers to the rapidly developing signs of focal and at times global loss of cerebral function with symptoms lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin (adapted from Hatano, 1976). Atherothromboembolism is by far the most

common cause of ischaemic stroke, with haematological disorders, trauma, and a variety of miscellaneous disorders the cause of the remainder (Lindley & Warlow, 1994).

2.2.3 Atherosclerosis

Atherogenesis is the formation and development of atherosclerotic lesions on the surface of arteries; it generally involves a sequence of reactions that may take several years to become clinically overt (Ross, 1986). It begins in early life but the complications of coronary heart disease do not usually occur until middle age (Stehbens, 1990). The term arteriosclerosis actually encompasses any condition of arterial vessels that results in a thickening and /or hardening of the walls (Price & Wilson, 2003).

2.2.4 Dietary fibre

Dietary fibre is defined as complex polysaccharide polymers that derive from plant cell walls which are resistant to the action of our digestive secretions (Kritchevsky, 1994). Dietary fibre is a generic term covering a number of substances of unique chemical composition and varying physiological effects. In the colon, fibre is broken down, by fermentation, to methane, hydrogen, carbon dioxide, and the respective short-chain fatty acids (SCFAs), principally acetic, propionic, and butyric acid. SCFAs are also known as volatile fatty acids. These acids are readily absorbed by the intestinal mucosa and metabolised by body tissue (Kritchevsky, 1994).

2.2.5 Risk factor *versus* risk marker

Risk factor represents those variables which are considered to contribute to the chances of developing CHD, for example diet and diabetes mellitus. While, risk marker represents those variables which are measured in the patient's blood that are associated with increased risk for developing CHD and stroke, for example fibrinogen and cholesterol.

2.3 EPIDEMIOLOGY

Cerebrovascular disease and coronary heart disease are important causes of morbidity and mortality in the western world (Renaud & Lorgeril, 1992) and amongst South Africans (Bradshaw *et al.*, 1995). Cardiovascular disease is the prime cause of death among the South African white, coloured and Indian populations, and the third most common cause of death among black South Africans aged 15 - 64 years (Bradshaw *et al.*, 1992). Although blacks still have the lowest cardiovascular disease mortality rate in South Africa, a survey reveals a deteriorating pattern for cardiovascular risk (Steyn *et al.*, 1991, Mollentze *et al.*, 1995), because of exposure to ongoing demographic transition and aggressive marketing of unhealthy consumer products. Steyn *et al.* (1992) reported that in 1988 ischaemic heart disease and cerebrovascular disease accounted for 9.6% and 7.9% respectively of deaths of all South Africans aged 35 - 64 years. This indicates a loss in the most productive sector of the labour force and

clearly constitutes a major cost to the economy. Increasing urbanisation, the adoption of a westernised lifestyle and concurrent emergence of associated diseases in black South Africans seem set to further increase the national cost of cardiovascular disease. The other reason for the deteriorating pattern of risk is the lipodystrophy associated with HIV antiretroviral treatment (Law *et al.*, 2003; Miller *et al.*, 2003). The link between antiretroviral treatment and coronary heart disease is not dealt with in this dissertation; it is only mentioned to illustrate the future impact.

2.4   RISK MARKERS FOR ATHEROSCLEROSIS AND THROMBOSIS

The aetiology of ischaemic heart disease appears to have two components: a thrombotic component and a dietary fat/blood lipid component (Packham & Kinlough-Rathbone, 1994). Any agent that damages or removes the endothelium can initiate or exacerbate atherosclerotic lesions, particularly when circulating cholesterol levels are high. Injurious agents may synergize with each other. Some of the agents that have been shown to damage the vessel wall also increase the sensitivity of platelets to aggregating agents, or activate the coagulation mechanisms, leading to the formation of thrombi. In the absence of an unequivocal ante mortem diagnosis, factors which have been identified epidemiologically with the risk of coronary heart attack are examined.

Thus the haemostatic process; followed by the haemostatic, lipid and other risk markers will be discussed in the next section of this chapter.

2.4.1 The haemostatic process

Haemostasis, the spontaneous arrest of blood from ruptured vessels, involves the interaction of the damaged vessel wall, the blood platelets, and circulating blood coagulation factors that form a localised stable mechanical seal that subsequently undergoes slow removal by fibrinolysis (Ratnoff & Forbes, 1996), as depicted in Figure 2.1.

VASCULAR INJURY

exposed
subendothelium

exposed
tissue factor

Vasoconstriction   Platelet aggregation ⟷ Coagulation

Clot formation

Fibrinolysis
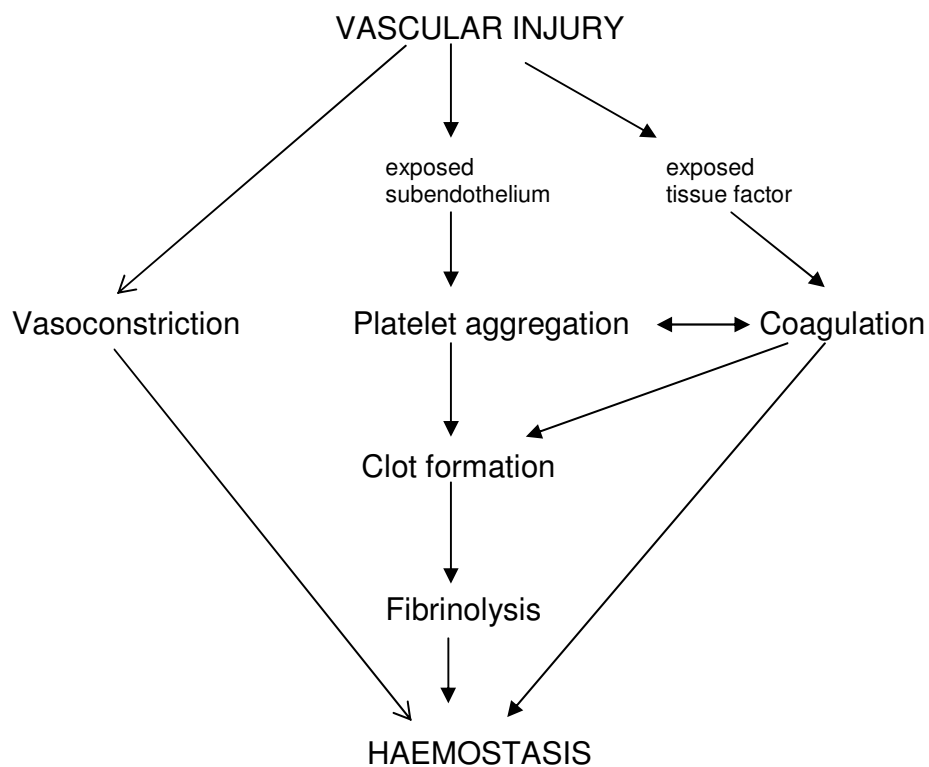
HAEMOSTASIS

Figure 2.1 Overview of haemostasis, adapted from Ratnoff & Forbes (1996).

The haemostatic system is designed to maintain blood in a fluid state under physiological conditions but primed to react to vascular injury in an explosive manner to stem blood loss by sealing the defect in the vessel wall (Ratnoff & Forbes, 1996). Rapid, localised haemostasis is achieved by complicated systems of activation and inhibition whereby excessive bleeding and unwanted thrombosis is minimised (Hutton *et al*., 1999). The explosive cellular and molecular reaction is modulated by endothelial cell elaboration of antithrombotic lipids, proteins, inorganic compounds and several plasma proteases (Kemball-Cook *et al*., 2004).

2.4.1.1 <u>Vascular endothelium</u>

Normal vascular endothelium maintains blood fluidity by producing inhibitors of blood coagulation and platelet aggregation, modulating vascular tone and permeability, and by providing a protective envelope that separate haemostatic blood components from reactive sub-endothelial structures (Kemball-Cook *et al*., 2004). It modulates fibrinolysis by synthesising and secreting tissue plasminogen activators and plasminogen activator inhibitors (Ratnoff & Forbes, 1996). Damage to a blood vessel exposes the elastin and collagen fibres in the supporting external layers that provide the principle stimulus. Endothelial disruption activates all four components of the haemostatic apparatus: rapid vasoconstriction, platelets adherence and aggregation, coagulation and fibrinolysis (Hutton *et al*., 1999).

2.4.1.2 <u>Platelets</u>

Platelets do not adhere to normal vascular endothelial cells. However, an area of endothelial disruption provides binding sites for the adhesive proteins (Watson & Harrison, 2004). Platelets adhere first to the basal layer of adherent platelets and eventually to one another, forming a mass of aggregated platelets (Hutton *et al.*, 1999). There is also release of platelet content that potentiates platelet aggregation. During activation, platelets expose receptors for specific plasma clotting factors, particularly activated factor V (Va), which may be either secreted and expressed by platelets or bound from plasma. This 'acquired' receptor in conjunction with anionic phospholipids exposed on activated platelets provides an efficient surface for coagulation (Hutton *et al.*, 1999).

2.4.1.3 <u>Coagulation</u>

The process of coagulation occurs in a series of complex steps, involving various coagulation enzymes and factors, which terminate in the formation of the blood clot (Kemball-Cook *et al.*, 2004). Although it has been traditional to divide the coagulation system into intrinsic and extrinsic (tissue factor) pathways, such a division does not occur *in vivo.* The principal initiating pathway of *in vivo* blood coagulation is the tissue factor system, which involves components from both the blood and

vascular elements (Ratnoff & Forbes, 1996), as summarised in Figure 2.2.
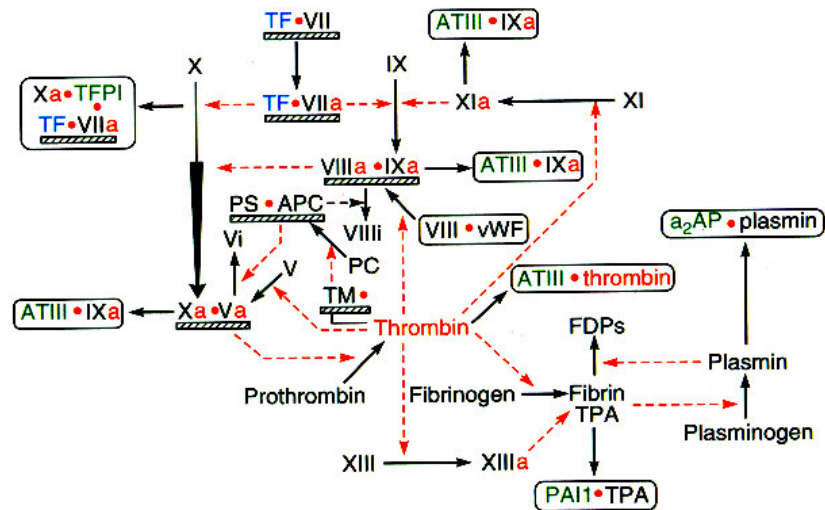
Figure 2.2 Integrated *in vivo* haemostasis. All these processes, plus platelet activation occur simultaneously, with a short lag time. Inhibited complexes are shown in cartouches; dotted lines = proteolytic activation/inactivation; solid arrows = change of state; and the hatched underline = phospholipid surface upon which activator complexes assemble (Hutton *et al.*, 1999).

2.4.1.3.1 *Fibrinogen*

Fibrinogen as a soluble glycoprotein has a molecular weight of 340,000 Daltons and is present in high concentration in both plasma and platelet granules (Hutton *et al.*, 1999).   The fibrinogen gene cluster is located on chromosome 4 q 31 in the order γ α β with β transcribed in the opposite direction to γ and α. The overall structure of fibrinogen is a symmetrical dimer α_2 β_2 γ_2 (Kemball-Cook *et al.*, 2004).   The molecule is trinodal with the outer two globular domains

(fragment D) containing the carboxytermini of all three chains, connected to the central globular domain (fragment E), which contains the N-termini (where fibrinopeptide A and B are found) of all the 6 chains tethered together by disulphide bonds. The lateral and central globular domains are connected by soiled coil region, forming α-helical ropes (Hutton *et al.*, 1999).

2.4.1.3.2 *Fibrin network formation*

The precursor of fibrin is fibrinogen, which interacts with other proteins, including factor XIII, fibronectin, α_2-antiplasmin inhibitor, plasminogen, and plasminogen activator (Hutton *et al.*, 1999). Polymerisation of fibrinogen occurs when thrombin, which binds to the central domain, cleaves two short negatively charged fibrinopeptides A and B from the N-termini of the α and β chains respectively, resulting in the formation of a transient intermediate, termed fibrin monomer (Hutton *et al.*, 1999). Fibrinopeptide A is released more rapidly from fibrinogen than fibrinopeptide B, but as polymerization to protofibrils proceeds, the rate of fibrinopeptide B release is increased nearly sevenfold (Kemball-Cook *et al.*, 2004). This reveals new N terminal sequences in the fragment E region (called knobs) which fit into holes in the fragment D regions. Polymerisation then occurs spontaneously in a half overlap array, which can elongate indefinitely in either direction (Ratnoff & Forbes, 1996).

Release of either set of fibrinopeptides initially leads to a half-staggered overlap structure. The structure is stabilised by non-covalent interactions between complementary polymerisation sites present on the central and outer domains of the fibrin (Kemball-Cook *et al.*, 2004). Continuing thrombin-catalysed cleavage of the fibrinopeptides from the central domain of fibrinogen leads to the formation of two-stranded polymers of fibrin, termed protofibrils, in a rapid bimolecular polymerisation process. Protofibrils' additional stability is provided by non-covalent interactions between the distal D domains of fibrin molecules aligned end-to-end in the same strand of each protofibril (Ratnoff & Forbes, 1996). Progressive lengthening of the polymer chain occurs by a half-overlap, side-to-side approximation of fibrin monomer molecules, and the two-stranded protofibrils interact laterally to form either long, thin fibrin strands or short, broad sheets of fibrin. In mature forms the fibrin fibre contains about 100 protofibrils, with a somewhat random pattern of branching that links the fibres together (Ratnoff & Forbes, 1996).

It is suspected that the degree of lateral strand association probably contributes to the tensile strength of the clot. Clot resistance to plasmin degradation is believed to be influenced mainly by crosslinking, mediated by Factor XIII (Doolittle, 1994). In the early stages of the fibrin assembly process, protofibrils are stabilised by the factor XIIIa-catalysed formation of crosslinks between the γ-chains (D

domains) of the assembling fibrin molecules (Doolittle, 1994). After pairing of virtually all the γ-chains in the fibrin network, a slower process of multiple crosslink formation between α-chains proceeds. These interwoven α-chain bonds are probably more critical for clot lysis resistance than is the limited geometry of the γ-γ crosslink. Factor XIIIa also covalently crosslinks α_2-antiplasmin, the principal fibrinolytic inhibitor, to α-chains in the clot, thereby increasing resistance to degradation (Ichinose, 1994).

2.4.1.4 <u>Fibrinolysis</u>

The fibrinolytic system removes unwanted fibrin deposits to re-establish flow in vessels occluded by a thrombus and to facilitate the healing process following inflammation and injury (Hutton *et al.*, 1999). Although only a small proportion of plasma plasminogen is bound to fibrin during particulate clot formation, this is sufficient to influence subsequent physiologic breakdown of the clot (Kemball-Cook *et al.*, 2004). The process is a balanced one, involving plasminogien activator inhibitor-1 and α_2 -antiplasmin (Gaffney & Longstaff, 1994).

2.4.2 Haemostatic risk markers and factors

Hypercoagulability can be broadly defined as a risk of thrombosis in circumstances that would not cause thrombosis in a normal subject (Schafer, 1994). From a clinical standpoint, patients suspected of having

a hypercoagulable state can be divided into two broad categories (Schafer, 1994). The first category is the primary hypercoagulable state, with a specific defect in heparin-antithrombin III, protein C-thrombomodulin and protein S, or plasminogen-plasminogen activator mechanisms (Walker, 2004). The second category consists of a heterogeneous array of clinical disorders in which there is an apparent increased risk for developing thrombotic complications as compared to the general population, for example, lupus anticoagulant, diabetes mellitus and obesity (Schafer 1994).

2.4.2.1 <u>Fibrinogen as a risk factor for CHD and CVD</u>

Prospective epidemiological studies have reported strong positive relationships, between fibrinogen level and the risk of myocardial infarction and/or stroke (Wilhelmsen *et al.*, 1984, Stone & Thorp, 1985, Meade *et al.*, 1986, Kannel *et al.*, 1987, Heinrich *et al.*, 1991, Møller & Kristensen, 1991). The authors concluded that a high fibrinogen level was an independent risk factor for cardiovascular disease and that fibrinogen plays an especially important role in the thrombotic process, thus in the pathogenesis of cardiovascular disease (Figure 2.3). It should be taken into account that the thrombotic tendency is unlikely to be accounted for by a single component of the haemostatic cascade, due to the complex pathophysiology of thrombosis. Many studies have found a strong positive association between smoking and fibrinogen level, with

smokers having the highest fibrinogen levels, moderate levels among ex

smokers and the lowest levels in non-smokers (Kannel, 1997;

Wilhelmsen, 1988; Møller & Kristensen, 1991).
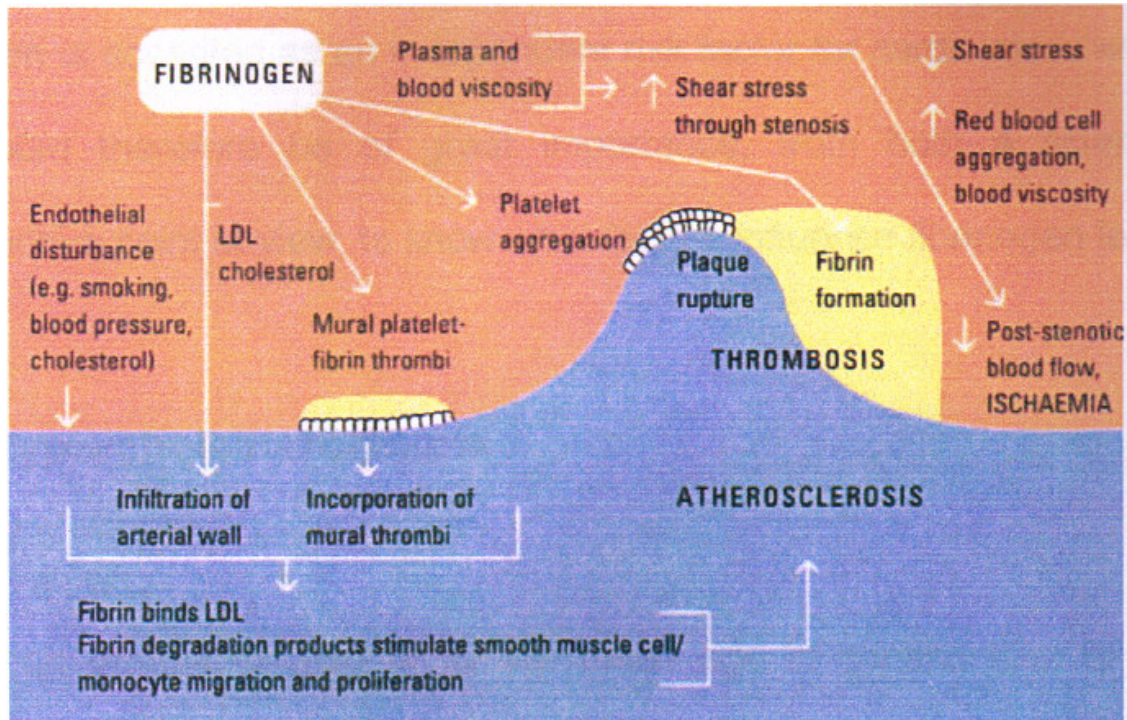
Figure 2.3 Summary of potential mechanisms by which increased plasma fibrinogen levels may promote arterial disease and ischaemic events. LDL=low density lipoprotein (Lowe, 1993)

2.4.2.2 Fibrin network architecture

It is suspected that not only the fibrinogen concentration but also the

quality of fibrin networks may contribute to CHD risk (Blombäck *et al.*,

1992). It is found that fibrin clots composed of dense fibre networks are

found in young coronary artery disease patients (Mills *et al.*, 2002) and

that their healthy male counterparts have clots with thicker fibres. Fibrin

networks formed from plasma of the healthy controls are more permeable (Mills *et al.*, 2002).

Vascular occlusive disease has a higher incidence in the diabetic patient compared to the non-diabetic (Ostermann & van de Loo, 1996). Nair *et al.* (1991) found that in the diabetic patient the fibre thickness (measured as the ratio of mass-length of the fibres) and permeability of networks are reduced. These networks are also more resistant to lysis.

It is known that any given network comprises of a major network of thicker fibres and a minor network of thinner fibres. The minor network occupies the interstitial spaces between the major fibres (Nair *et al.*, 1986). According to Blombäck *et al.* (1992) kinetic and modulating factors determine the dimensional gel structures. Kinetic factors include the thrombin and fibrinogen concentration, while modulating factors include proteins and ions in close contact with the fibrinogen molecule (Blombäck *et al.* 1992).

Three independent methods are used to characterize the fibrin network architecture. The mass-length ratio serves as a measure of fibrin thickness (Nair *et al.*, 1991). Permeability serves as a measure of variability in fibrin fibre thickness and pore size within the network, which indicates the degree to which fluids can move through the network and therefore carry enzymes that support the breakdown of the network, especially of unwanted clots (van Gelder *et al.*, 1995). Compaction characterises the degree of cross-linking and tensile properties of the

fibrin network (Nair & Shats, 1997). Other techniques include the network lysis rate, which could be used to assess the overall structure of the fibrin network in terms of lytic rate under controlled conditions. Gabriel *et al.*, (1992) have shown that thin fibrin fibres are lysed more slowly when compared to thick fibrin fibres.

It is suggested that fibrin networks with mostly thin fibres, decreased permeability and higher tensile strength are more atherogenic in nature (Blombäck *et al.*, 1992). Fibrin networks with mostly thicker fibres, which are more permeable and have lower tensile strength, are believed to be less atherogenic. This is because they are more deformable, allow easy access of the lytic enzymes and also have greater exposed surface areas for the fibrinolytic agents (Blombäck *et al.*, 1992).

2.4.2.3 <u>Factor VII</u>

A high blood factor VII activity level has been reported to predispose patients to the onset of ischaemic heart disease (Meade *et al.*, 1986, 1986a). Surgue *et al.* (1985) demonstrated that in a series of patients with heterozygous familial hypercholesterolaemia, the age adjusted mean plasma fibrinogen level and factor VII activity were significantly higher in patients with evidence of CHD when compared to patients without CHD. Serum lipids concentration did not differ significantly between the two groups. Heinrich *et al.* (1994) reported that there is a trend toward higher factor VII activity and occurrence of CHD events.

Meade *et al.* (1986) postulated that dietary fat may be an important determinant of factor VII activity. Miller *et al.* (1986) found a positive correlation between dietary cholesterol intake and factor VII activity. Factor VII is assumed to be activated *via* the negative charge of chylomicrons and VLDL. Accordingly, dietary intake of fats could influence the risk of ischaemic disease *via* a long term atherogenic and a short-term thrombogenic pathway. Since factor VII activation leads to an increase in thrombin concentration, thereby intensifying platelet activation and procoagulatory mechanisms, it can be assumed that, conversely, cholesterol-lowering measures also reduce the thrombotic risk associated with a high activity of factor VII (Miller *et al.*, 1986).

2.4.2.4 Factor VIII

Since factor VIII and von Willebrand factor are both procoagulants, favouring clot formation and platelet adhesion, high levels might be expected to be risk factors for thrombosis and atherosclerosis. Conlan *et al.* (1993) showed a strong association of factor VIII with a number of known cardiovascular disease risk factors. Pan *et al.* (1997) found a positive association between factor VIII concentration and carotid atherosclerosis in the Chinese population. The authors suggest that the association might not be independent. In the Northwich Park Heart Study, the individuals in the highest tertile group of factor VIII levels were associated with a 44% increase in risk for CHD (Meade *et al.*, 1986).

Meade *et al.* (1986a) found that factor VIII was highest in Africans than Caucasians as observed in other studies (Conlan *et al.*, 1993). It is suggested that factor VIII levels may be determined predominantly by genetic (ethnic group and ABO status) rather than environmental characteristics.

2.4.2.5 Fibrinolytic factors

A variety of fibrinolytic defects have been implicated in the pathogenesis of thrombosis. These include decreased release of tissue plasminogen activator (Nilsson *et al.*, 1985) and increased plasminogen activator inhibitor -1 (PAI-1) concentration (Hamstern *et al.*, 1987). The net effect of each of these abnormalities is impaired functional plasmin generation and a blunted fibrinolytic response to fibrin formation (De Pergola & Pannacciulli, 2002). High PAI-1 levels may be markers that help identify individuals with an increased risk of recurrent myocardial infarction (Hamstern *et al.*, 1987).

2.4.2.6 Platelets as a risk factor for CHD and CVD

The development of thrombosis, especially arterial, is greatly influenced by platelets. This is important where vessel disease, especially atherosclerosis, is the underlying cause. Platelet hyperaggregability is associated with increased risk of cardiovascular incidence (Mammen, 1999). This is particularly true for sticky platelet syndrome, where

platelets show marked hyperaggregability with ADP and/or epinephrine (Mammen, 1995; Frenkel & Mammen, 2003). These patients have an increased risk of developing thrombosis, even while on anticoagulant therapy or with no evidence of other hyperaggregability conditions.

2.4.3 Lipid risk markers

A total plasma cholesterol level above 5.2 mmol/l is considered to promote the development of advanced atherosclerotic lesions (Roberts, 1989; Oliver, 1990). The Lipid Research Clinics Coronary Primary Prevention Trial (1984 a & b) and Helsinki Heart Study (Frick *et al.*, 1987), clearly showed that lowering plasma cholesterol levels brings benefit in terms of reduction of coronary morbidity. High cholesterol levels have also been reported to result in platelet hypersensitivity to aggregating agents in man (Bradlow *et al.*, 1982; DiMinno *et al.*, 1986). The question of whether elevated levels of plasma triglycerides are an independent risk factor for coronary heart disease is unsettled and controversial (Oliver, 1990; Austin, 1991; Criqui, 1991; Wilson *et al.*, 1991). It is hypothesised that the risk for coronary artery disease associated with blood cholesterol increases with rising plasma concentrations of LDL-C. However, it is also believed that the risk associated with LDL-C is diminished when associated with high HDL-C concentrations. For this reason, some laboratories calculate the ratio of LDL-C: HDL-C for estimating the degree of risk; a ratio of less than

3:1 is considered desirable (Kaplan *et al.,* 1995). The LDL:HDL ratio is a strong predictor of cardiac events (Hermansen *et al.*, 2003).

2.4.3.1 <u>Lipoprotein classes</u>

Cholesterol, plasma phospholipids, triglycerides and other hydrophobic fats, enter and leave plasma bound to specific proteins, forming lipoproteins (Kaplan *et al.*, 1995). The protein and phospholipids impart solubility to the otherwise insoluble lipids. Apolipoprotein (apo) are protein moieties of the lipoprotein. The lipoproteins act as carriers of triglyceride. Lipoproteins can be classified as chylomicrons, very-low density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), the low-density lipoprotein (LDL), or high-density lipoprotein (HDL). Each of these lipoprotein families contains cholesterol, but in differing proportions. Chylomicrons, for example, which are more than 95% triglyceride by weight, carry 1% cholesterol and less than 1% protein. VLDL, in contrast, contains about 10% protein, 18% cholesterol by weight, and has fivefold more triglycerides compared to cholesterol. LDL contains 50% cholesterol by weight; while HDL has 50% protein by weight and an estimated 23% cholesterol content (Kaplan *et al.*, 1995).

2.4.3.2 <u>Low-density lipoprotein cholesterol (LDL-C)</u>

LDL-C binds to specific primary receptors on fibroblasts, which are responsible for their transport into the cell (Levy, 1981). In humans the concentrations of LDL-C in the bloodstream totally saturate the primary LDL-C receptor sites *in vivo*. Other non-specific receptors become involved in the clearance of lipoprotein - including receptors on the lining of the blood vessels themselves, where cholesterol accumulates. It is postulated that the reason humans are susceptible to atherosclerosis is because our LDL-C concentrations are higher and above the threshold of primary clearance (Levy, 1981).

Two main LDL-C phenotypes have been identified, one characterised by the predominance of large buoyant LDL-C particles and other by an excess of small, dense LDL-C particles. A pattern consisting of an increased concentration of small, dense LDL-C particles, an elevation of triglycerides and a reduction in HDL-C has been shown to increase the risk of ischaemic cardiac events (Halle *et al.*, 1996).

2.4.3.3 <u>High density lipoprotein cholesterol (HDL-C)</u>

There exists a negative association between HDL-C and the incidence of ischemic heart disease (Stampfer *et al.*, 1991). A circulating HDL-C concentration of less than 35 mg/dl (approximately 0.85 mmol/L) is believed to promote the onset of atherogenesis, while a higher concentration of the molecule is believed to offer protection against

atherosclerosis. HDL-C is not monodisperse, but exists as two main populations, designated HDL_2 and HDL_3. Of the two, HDL_3 is major and HDL_2 minor in mass terms, but not in clinical significance (Cheung *et al.*, 1991).

2.4.3.4 <u>Dietary fat and lipid metabolism</u>

The interplay between diet and lipid metabolism has been the topic of investigation for many decades. Both randomised clinical trials and long-term epidemiological studies were used to investigate possible relationships between dietary fat intake and lipid metabolism. Diets rich in saturated fatty acids of chain length 12 to 16 carbons not only raise circulating total cholesterol levels (Ulbright & Southgate, 1991), but have also been shown to be thrombogenic in rats and in man. It is believed that polyunsaturated fatty acids are antithrombotic in nature (Hornstra, 1980, 1989; NordØy & Goodnight, 1990). It has been pointed out that the ratio of polyunsaturated to saturated fatty acids (P/S ratio) in the diet also influences platelet function in man (Renaud, 1990). Renaud (1990) noted that a dietary P/S ratio of 0.7 is characteristic of the Japanese diet, a population in whom the clinical complications of atherosclerosis are reduced. Enrichment of diets with omega-6 polyunsaturated fatty acids has been shown to depress serum-HDL-C levels, as well as lowering the LDL-C (Ulbright & Southgate, 1991). Trans-polyunsaturated fatty acids (produced during the hydrogenation of liquid vegetable oils), in contrast

to cis-polyunsaturated fatty acids, have been found to raise LDL-C levels and lower HDL-C levels in man (Mensink & Katan, 1990), and hence are considered to be atherogenic. A diet low in saturated and trans-fatty acids, with adequate amounts of monounsaturated and polyunsaturated fatty acids, especially long-chain omega-3 fatty acids, would be recommended to reduce the risk of developing CHD (Lichtenstein, 2003).

2.4.4 Other cardiovascular risk factors

Other risk factors generally associated with increased cardiovascular risk, include:

- Smoking status (Hennekens *et al.*, 1984; Haapanen *et al.*, 1989; Homer *et al.*, 1991; Ingall *et al.*, 1991),

- Obesity (Lowe *et al.*, 1988; Lee *et al.*, 1990; Lowe *et al.*, 1992; De Pergola & Pannacciulli, 2002),

- Diabetes mellitus (Colwell, 1988; Ostermann & van de Loo, 1996),

- Hypertension (Kannel, 1991),

- Stress, or type A personality (Siltanen, 1987; Dimsdale, 1988),

- Adrenaline and noradrenaline (Hourani & Cusack, 1991),

- Hyper-homocysteinaemia (McCully, 1990; Clarke *et al.*, 1991),

- Gender (Beard *et al.*, 1989; Stampfer *et al.*, 1991a),

- Physical inactivity (Powel *et al.*, 1987; Møller & Kristensen, 1991; Kannel, 1997) and

- Age (Bush *et al.*, 1988; Witterman *et al.*, 1989; Barrett-Connor & Bush, 1991).

2.5   PROPOSED DISEASE MECHANISMS

Stroke and coronary heart disease are multifactor diseases. Their sequelae encompass genetic factors; physiological factors, such as metabolism of the arterial wall; humoral factors, including lipids and lipoproteins and the complex mechanics of blood clotting; stress and psychological factors; and ecological factors which include diet and behaviours such as cigarette smoking (Ulbright & Southgate, 1991). Stroke and CHD commonly involve atherosclerosis and thrombosis thus the following section will be used to supply a detailed description of thrombogenesis and atherogenesis.

2.5.1 Thrombogenesis

A thrombus is a deposit formed from blood constituents on the surface of the heart lining or blood vessels, and can occur anywhere in the circulation (Summers *et al.*, 2004). In 1856 Virchow proposed that three major factors determine the site, kind and extent of a thrombus: first is the mechanical effect in which blood flow is predominant; second is alterations in the constituents of blood; and thirdly, changes in the vessel wall

(Packham & Kinlough-Rathbone 1994). Thrombosis and blockage of narrowed arteries is a fatal event and the cause of more than 90% of deaths from CHD (Polk, 1985). Thus, the partially occluded artery is easily blocked by thrombi formed from aggregated platelets or white blood cells that rapidly aggregate and clump in response to specific stimuli (Polk, 1985; Ross, 1986; Kinsella *et al*., 1990).

Vascular damage initiates activation of platelets and the coagulation cascade that is fundamental to thrombus formation. Aggregation is induced by thromboxane A_2 (Kinsella *et al*., 1990). Arterial thrombi are stabilised by the following:

- adhesive blood proteins

- the granules of platelets that have released their granule contents,

- ADP from platelet dense granules or from red blood cells, and

- by fibrin formed under the influence of thrombin.

In addition, clot retraction mediated by platelet actomyosin consolidates a thrombus and makes it less susceptible to fibrinolysis (Fitzgerald, 1991).

2.5.2 Atherogenesis

Atherosclerotic lesion formation can be induced in two ways: by enriching the diet with cholesterol (or saturated fats) (Armstrong & Heistad, 1990) or by repeated injury of the wall of the artery of normocholesterolaemic man or animals (Tyson *et al*., 1976; Moore, 1981). The theory of repeated

injury is supported by re-occlusion of vessels and accelerated development of atherosclerotic lesions after procedures such as bypass surgery, angioplasty and endarterectomy (Summers *et al.*, 2004). Injury-induced atherosclerosis is exacerbated by lipid-enriched diets (Minick, 1981). Atherosclerosis is a complex process so it is believed that both theories might play a role simultaneously.

The suggested sequence of events is that monocytes adhere to the endothelium, migrate into the intima, and become macrophages that take up lipids. Before uptake, low-density lipoprotein cholesterol (LDL-C) is oxidised, either in or adjacent to endothelial cells, or when it is bound to proteoglycans in the intima. Furthermore, it is shown that extensive oxidation of LDL-C leads to its aggregation (Moar *et al.*, 1997). Both of these modified forms of LDL-C are present in the atherosclerotic lesion (Aviram *et al.*, 1995). Following infiltration, macrophages phagocytose the cholesterol rich LDL-C particles (especially when oxidised or modified), mediated by scavenger receptors on the macrophage membrane (Aviram & Fuhrman, 1998). Foam cells are formed. The macrophages secrete growth factors, thereby stimulating the proliferation and migration of smooth muscle cells (Steinberg, 1988, 1992; Esterbauer *et al.*, 1992). Other sources of growth factors include the endothelial cells and the smooth muscle cells (Ross, 1989; Gordon *et al.*, 1989; Clowes, 1991). Over time, these lipid-laden cells progressively accumulate and the adjacent smooth muscle cells proliferate, causing the formation of a raised

lesion (Ross, 1986; Steinberg, 1988; Kinsella *et al.*, 1990). Eventually, the endothelium is disrupted over the foam cells. At this stage platelets adhere and are induced to secrete growth factors, which further stimulate the migration and proliferation of smooth muscle cells (Faggiotta & Ross, 1984; Masuda & Ross, 1990). Gradually the lesion develops into a fibrous plaque. The calcification of advanced lesions may occur. Ultimately, plaque impedes blood flow. There is growing evidence that oxidation and/or modification of the lipids contained within LDL-C greatly increase the capacity of macrophages, endothelial cells, and monocytes to bind and ingest oxidised LDL *via* receptors, thus accelerating atherogenesis (Ross, 1986; Steinberg, 1992).

2.6 DIETARY FIBRE

In 1885 Louis Pasteur suggested that the presence of bacteria in the alimentary tract was essential for the life of rabbits and guinea pigs, although this suggestion was subsequently disproved by the successful rearing of germ-free specimens of these animals. Their shortened survival supported the principle that the intestinal flora is important for normal nutrition (Wrong, 1995).

Dietary fibres from natural or semi-synthetic sources have gained increasing attention because of their value as a supplement to the normal

western diet, which is poor in bulking substances. Addition of fibre for treatment of disorders such as atherosclerosis and colon cancer could hold therapeutic value (Wrong, 1995).

2.6.1 Short chain fatty acids (SCFAs)

Short chain fatty acids are the end product of microbial fermentation within the digestive tract. Those of interest to us can be described as "saturated unbranched alkyl monocarboxylic acids of 2 - 4 carbon atoms" (Wrong 1995). SCFAs (acetate, propionate & n-butyrate) and gases (H_2, CH_4 & CO_2) are the main end products of the anaerobic breakdown of complex polysaccharides by colonic bacteria (Bourquin *et al.*, 1992a). SCFAs make up the predominant anions in the large intestine of mammals and create a slightly acidic pH level (6.0-7.0). SCFAs vary widely in their relative proportions, depending upon the fibre source in the diet. SCFAs in human faeces, following consumption of different defined polysaccharides, have been measured on average, in the molar ratio of acetate: propionate: butyrate of 53: 27:20 (Brøbech-Mortensen & Clausen, 1996).

2.6.2 Fermentation

"Fermentation" in the human large intestine is used to describe the great variety of reactions and the overall metabolic process involved in the anaerobic breakdown and partial mineralization of organic matter, catalysed by microbial enzymes (Macfarlane & Gibson, 1995). The

microbial populations in the large intestine are composed of several hundred different micro-organism species (prokaryotes and eukaryotes). In general, most individuals within the same racial and socio-economic groups harbour similar cell population densities and generic distribution of bacteria in their colons (Macfarlane & Gibson, 1995).

Substrate for microbial fermentation in the large intestine are macromolecules that come from endogenous (sloughed epithelial cells, lysed microbial cells, mucus, and other intestinal excretion) and exogenous (mostly fibrous components of food) sources, that cannot be digested by host enzymes (Brøbech-Mortensen & Clausen, 1996). The major substrates include polysaccharides, non-starch polysaccharides from plant cell walls, dietary proteins and peptides (Macfarlane & Gibson, 1995). Dietary fibres are complex polysaccharide polymers that derive from plant cell walls. The structure is made up of water-insoluble cellulose microfibrils coated with soluble hemicellulose and embedded in a gel of pectins. Variable amount of starch that escape digestion in the small intestine (this fraction is called resistant starch) also pass into the colon and become available as substrate for microbial fermentation, as does dietary fibres (Brøbech-Mortensen & Clausen, 1996).

The more predominant factors that affect SCFA production from polysaccharides and protein by bacteria in the large intestine are as follows:

- chemical composition of the fermentable substrate;

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- amount of substrate available;

- type of bacteria present in the large gut;

- rate of depolymerization;

- colonic transit time;

- availability of inorganic electron acceptor;

- pH of the gut content, particularly in the proximal colon;

- fermentation strategies of substrate-utilising bacteria;

- substrate specificities and catabolite regulation mechanism of individual competitive and co-operative particle size;

- solubility and association with indigestable complexes such as lignins, tannins and silica (Macfarlane & Gibson, 1995).

2.6.3 Absorption

There is ample evidence that SCFAs are rapidly absorbed from the colonic lumen. Cummings (1981) suggested that, on the basis of a typical western diet containing 20g fermentable carbohydrate, 200 mmol SCFA are produced by colonic anaerobic bacteria; 90-97% (180-193 mmol) of SCFA are absorbed and only 3-10% (7-20 mmol) are excreted in faeces (Scheppach *et al.*, 1987; Brøbech-Mortensen & Clausen, 1996). Several models have been proposed to explain SCFA transport (Engelhardt, 1995) and to account 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 mucosa. SCFAs may also be transported in the ionised form *via* an SCFA- HCO_3^- exchange mechanism.

SCFA absorption in the large intestine involves several processes: non-ionic diffusion, carrier-mediated mechanisms, diffusion after protonation, carrier mediated SCFA anion exchange with bicarbonate, and diffusion coupled with sodium absorption (Scheppach *et al.*, 1987). It is widely believed that 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 are needed for SCFA protonation, because 99% of SCFA (pKa = 4.8) are in the ionised form for hydration of luminal CO_2 to HCO_3^- and H^+. In addition, the mechanism of uptake into enterocytes is undoubtedly a complex process involving a number of factors and mechanisms (Engelhardt, 1995). The precise mechanism for the absorptive process of SCFAs remains undefined, but all these above outlined factors seem to play a role (Brøbech-Mortensen & Clausen, 1996).

2.6.4 Metabolism

Butyrate is found in lower proportion in the blood draining the large intestine when compared to blood draining the lumen (Brøbech-Mortensen & Clausen, 1996). This reflects the fact that a large part of absorbed butyrate is metabolised by the mucosa (to carbon dioxide and ketone bodies). Butyrate is an important fuel for the colonic mucosa whereas

acetate and propionate are released into the portal blood. Portal vein concentrations of butyrate are approximately tenfold higher when compared to the peripheral vein concentration, while propionate concentrations are 20 - 40 folds higher and acetate concentrations only approximately fourfold higher. The major site of SCFA metabolism is the liver; where butyrate and propionate are almost entirely absorbed. However, the percentage of acetate uptake is lower (frequently less than 50%). SCFA concentrations in the portal vein are closely dependent on digestive fermentation (Brøbech-Mortensen & Clausen, 1996).

Hepatic butyrate uptake is practically total under physiological conditions. Butyrate is exclusively metabolised in mitochondria (carnitine-independent source of acetyl-CoA) and act as a potentially ketogenic substrate during the post-absorptive period. In fed subjects, butyrate may represent a precursor for lipogenesis. High concentrations of butyrate inhibit propionate utilisation. Because of the provision of acetyl-CoA in mitochondria, butyrate is an effective activator of gluconeogenis from lactate, and of ureogenesis; thus butyrate probably thwarts some of the inhibitory effects of propionate on gluconeogenesis (Rèmésy *et al.*, 1995).

Under healthy conditions, propionate is totally taken up by the hepatocytes. Propionate may depress the rate of ureogenesis, in contrast to acetate and butyrate that activate it. Propionate is also a very effective gluconeogenic substrate in most species. Propionate is probably a constituent precursor of glycogen synthesis and possibly of lipid synthesis.

It acts as an inhibitor of pyruvate oxidation in hepatocytes from fed rats. In addition, propionate increases the release of lactate in hepatocytes from fed rats by increasing glycolysis. This effect is counteracted by aleote, which suggests that propionate inhibits the production of acetyl-CoA and citrate (Rèmésy *et al*., 1995).

The metabolic fate of acetate and its role in the liver is connected to the general orientation of metabolism towards carbohydrate or lipid utilisation. Acetate stimulates *in vitro* fatty acid synthesis but inhibits cholesterol-genesis within isolated rat hepatocytes (Rèmésy *et al*., 1995). Furthermore, acetate can stimulate gluconeogenesis from lactate. A substantial fraction of acetate from digestive fermentation is not taken up by the liver. Splanchnic balance of acetate is always positive, even during fasting periods. Most of the extrasplanchnic tissues can metabolise acetate, for example, adipose tissue, mammary gland (cytosolic), muscle, kidney, and the heart (mitochondrial). These tissues have acetyl-CoA synthase. The cytosolic localisation of acetyl-CoA synthase should channel acetate toward lipogenesis, whereas the mitochondrial localisation favours its utilisation for energy supply in the Krebs cycle (Rèmésy *et al*., 1995).

2.6.5 Biological effects of SCFA

Various epidemiological and case control studies have shown that the intake of dietary fibre has some beneficial effects on the health of human subjects (Brøbech-Mortensen & Clausen, 1996). Especially the cholesterol-lowering effects of dietary fibre intake have been the topic of many studies. The specific mechanism through which dietary fibre decreases blood cholesterol concentration is unclear. Two possible hypotheses have been developed: (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 short-chain fatty acids, which are absorbed into the portal vein and attenuate hepatic cholesterol synthesis (Anderson & Siesel, 1990).

2.6.5.1 Butyrate

The effect of butyrate on haemostasis is not known, while the effect on lipid metabolism has not been extensively studied. In cultured cell lines, butyrate is a well-recognised antitumor agent, whereas the other SCFAs are much less active in this respect (Young & Gibson, 1995; Brøbech-Mortensen & Clausen, 1996).

2.6.5.2 Propionate

Venter *et al.* (1997) undertook a study where baboons were fed a western diet with either a 2% propionate or 5% soluble dietary fibre

concentrate supplement. Total serum cholesterol values were increased in the baboons fed an unsupplemented western diet. Soluble fibre supplementation prevented this increase, while propionate did not. However, both propionate and fibre intake increased the serum high-density-lipoprotein cholesterol concentration. Furthermore, the liver cholesterol concentration was lowered by propionate and fibre supplementation. Experimental studies in animals indicate that feeding oat bran has hypocholesterolaemic effects (Brøbech-Mortensen & Clausen, 1996). Concomitant to this, oat bran significantly increases portal vein propionate concentration. In comparison, cellulose that does not lower cholesterol levels does not increase the propionate concentration. *In vitro* studies indicate that propionate at physiological concentrations, significantly decreases hepatic cholesterol synthesis (Anderson, 1995). Other conflicting results show that propionate administered rectally, has no effect on either the circulating total cholesterol or the triglyceride concentration (Wolever *et al.*, 1991). When administered orally, propionate is reported to have no effect on serum cholesterol levels, but causes an increase in the triglyceride concentration (Brøbech-Mortensen & Clausen, 1996). Conflicting results show that the total serum cholesterol concentration is slightly decreased and the triglyceride concentration slightly increased (Anderson, 1995).

2.6.5.3 Acetate

When acetate is administered through different routes, for example oral, rectal and intravenous, to healthy human volunteers the following are found. Decrease in glycerol levels and a significant decrease in serum concentrations of free fatty acids. Acetate appears to compete with long-chain fatty acids for oxidation in certain tissues, promote hepatic cholesterol synthesis and decrease lipolysis (Topping & Pant, 1995). Veldman *et al.*, (1999) postulated that pectin and acetate effected a positive change in the fibrin network structure. These changes are partly accounted for by the direct effect of acetate on fibrin network structure.

2.7 SUMMARY OF LITERATURE REVIEW

Cerebrovascular disease and coronary heart disease (CHD) are of the most important causes of morbidity and mortality amongst South Africans. The risk factor prevalence for stroke and CHD becomes altered by changes in lifestyle, including diet. In general it is suggested that lifestyle management should be the first choice when having to treat patients with increased cardiovascular risk.

The prudent low-fat, high-fibre diet is regarded as an apparently healthy diet. It is suspected that this diet is effective for the control of known coronary risk factors as well as raised clotting factors. Research studies have shown the addition of dietary fibre to the diet as a promising

therapeutic agent for the limited control of known coronary risk factors. The physiological effects of dietary fibre in humans are significantly influenced by the degree to which fibre is fermented in the colon. Fermentation results in the production of short-chain fatty acids (SCFAs) acetate, propionate and butyrate.

This chapter has been used to show how CHD and stroke are related to lifestyle changes especially diet, and the reason why black people were chosen as the study population. CHD and stroke involves both haemostasis and lipids. Risk markers from both where discussed, to show a link between diet and these variables. Finally, the short-chain fatty acids' link with haemostatic and lipid risk markers was discussed to explore the possible mechanism by which SCFAs affects the risk markers. Thus a foundation has been laid for the understanding of this study

CHAPTER 3

MATERIALS AND METHODS

3.1   INTRODUCTION

In this study a group of westernised African male volunteers were recruited and fed two different combinations of short-chain fatty acids, as well as a placebo supplement, for a period of six weeks. Blood was drawn at different intervals and analysed to assess changes that were brought about by the different supplements. The study was explained to all volunteers, where they signed the Informed Consent Document (appendix A) and completed the recruitment questionnaire (appendix B). Blood sampling and medical examinations were performed at the medical facilities of the Tempe Military Base, Bloemfontein. Laboratory analyses of samples were performed at the Research Laboratory, School of Health Technology, Central University of Technology, Free State.

3.1.1 Ethical consideration

This study proposal was submitted to and approved by the Ethics Committee of the University of the Free State, reference number: ETOVS 227/98. The study was conducted according to International Good Clinical

Practice Guidelines and adhered to the contents of the Declaration of Helsinki.

3.1.2 Study design

Volunteers were recruited from the Military Base at Tempe Defence Force, Bloemfontein, and randomly assigned to three different supplementation groups. Group 1 received a placebo filled with non-fermentable cellulose. Group 2 received a supplement containing 50% acetate and 50% propionate. Group 3 received a SCFA supplement in the ratio of 70% acetate, 15% propionate and 15% butyrate. Neither researchers, field-workers nor the volunteers were aware of which supplement they were assigned to. The study was therefore double-blind.

A calculation based on the results of previous experiments was only of partial value for obtaining sample size since effects of propionate supplementation on outcomes measured in this study were not available. For power calculations a reference of 15% change in circulating blood cholesterol was used (Veldman, 2000). It was estimated that a group of 25 individuals per supplement would allow for expected drop-outs without a significant loss of statistical power.

3.1.3 Work schedule

A recruitment day was scheduled during which blood samples were drawn, dietary intake, social and geographic background questionnaires completed, as well as anthropometric measurements taken as part of the selection process. The scientific value of the study and expectations of the researchers were also explained to the volunteer, in both English and their preferred mother tongue. Inclusion criteria were strictly adhered to for selection of participants. A total of 75 subjects were selected after scrutinisation of the recruitment data. For standardisation purposes supplementation followed after a run-in phase of one week, during which all subjects consumed placebo supplements. Subjects were then randomly assigned to one of three groups. Blood was also drawn on day 8 (end of the run-in phase) which also served as a baseline measurement. Volunteers then consumed the pellets containing different combinations of SCFAs or placebo corresponding to their group assignment for a period of four weeks. A washout period of one week followed. Blood samples were drawn after two weeks of supplementation on day 22, and again at the end of the four week period on day 36. Following day 36, all subjects consumed placebo containing capsules for seven days, after which blood was drawn to assess the recovery of measured variables after supplementation. At the end of the study the subjects completed a questionnaire about their experiences during the study (appendix C).

3.2 MATERIALS

3.2.1 Study population

Only seventy-five subjects met the inclusion criteria. The subjects were permanent African male staff of Tempe Military base, Bloemfontein. The inclusion criteria were as follows:

- No smokers, alcoholics, and history of cardiovascular incidences

- No usage of chronic medications

- No usage of medication or other supplementation during the study, without the knowledge of the study leader

- No lifestyle changes during study and at least six months prior to the study

- Subjects should have been permanent staff for at least one year prior to the study

- No familial hypercholesterolaemic subjects were used

- Subjects should have a fibrinogen level of higher than 2.5 g/L in order to recognise significant effects on this variable.

- The background diet should be identical and contain about 15 grams unabsorbed carbohydrates.

3.2.2 Supplements

Volunteers obtained SCFA supplements in capsules equivalent to the fermentation of 15 grams dietary fibre. The capsules were designed in such a way that their contents were released only in the large intestine; they were resistant to stomach acid and small intestine enzymes (coated with a chelac-containing spray). The reason for this was to simulate the generation of SCFA in the large intestine. The supplements' content, ratio and concentration were calculated using the sheet attached in appendix D.

The acetate/propionate ratios were as follows:

Group one: Placebo   capsules containing indigestible cellulose.

Group two: Half + half   50% acetate 50% propionate 0% butyrate

Group three: High acetate   70% acetate 15% propionate 15% butyrate

Compliance of supplement consumption was monitored by the dieticians (Captains M De Wet & A van Onselen – who were involved in the study), as the entire subject group ate their meals at the Mess-hall of Tempe Military Base, Bloemfontein.

3.2.3 Specimen collection

Fasting samples were required thus venepuncture was performed between 8:00 and 10:00 am, and the subjects fasted from 22:00 the previous night. For full blood count and C-reactive protein analysis, blood was drawn into 5 ml EDTA tubes. Two 10 ml red top tubes were drawn for chemical analysis, and six full 5 ml tri-sodium citrate (3.8%) tubes were obtained for coagulation assays.

3.2.4 Apparatus

The apparatus used during the procedures were in good working condition and calibrated to give accurate results.

TABLE 3.1   The apparatus used during the procedures

Apparatus	Description	Brand name	Supplier
Coagulation analyser	Coagulation Timer	Berhring	Dade Berhring
Chemical analyser	Hitachi 902	Hitachi	Boehringer Mannheim
Cell counter	Coulter MD 18	Coulter	Beckman Coulter
Microplate reader	EL 312e	Biotek	Biotek instruments
CRP analyser	MICROS CRP	ABX CRP-100	ABX diagnostics
Spectrophotometer	Shimadzu UV-1201	Shimadzu	Scientific Group

3.2.5 Standards and controls

TABLE 3.2 Standards and controls used during variables' determinations

Standard or control	Catalogue no	Supplier
Fibrinogen Standards 1-4[#]	OWCS 11	Dade Berhring, Marburg, Germany
Standard Human Plasma[#]	ORKL 17	Dade Berhring, Marburg, Germany
Control Plasma N[#]	ORKE 35	Dade Berhring, Marburg, Germany
Control Plasma P[#]	OUPZ 13	Dade Berhring, Marburg, Germany
D-Dimer Standard Plasma[#]	OQXA 11	Dade Berhring, Marburg, Germany
D-Dimer Control Plasma I[#]	OQKA 15	Dade Berhring, Marburg, Germany
D-Dimer Control Plasma II[#]	OQKB 17	Dade Berhring, Marburg, Germany
S.F.A.C.*	759350	Roche Diagnostics, Mannheim, Germany
Percinorm® U*	171735	Roche Diagnostics, Mannheim, Germany
Percipath® U*	171760	Roche Diagnostics, Mannheim, Germany

[#] *the reagents used for calibration and control of coagulation assays, while * where used for chemical assays.*

3.2.6 Reagents and consumables

The reagents and some consumables which were used during the study are listed in the following table.

TABLE 3.3 Reagents and consumables used during the study

Reagents & consumables	Catalogue no	Supplier
Trasylol®/Aprotinin	H2912	Bayer-miles, Germany
Glass beads	267 02 50	Saarchem, South Africa
Sodium hydroxide	582 31 80	Saarchem, South Africa
Sodium carbonate	582 20 40	Saarchem, South Africa
Folin & Ciocalteu's reagent	243 300	Saarchem, South Africa
Albumin	T-3379	Sigma, USA St. Louis
Thrombin	101141	ICN, USA
Streptokinase	10114	ICN, USA
CRP latex reagent	7003077	ABX Diagnostics, France

3.3   METHODS

3.3.1 Specimen preparation

Full blood count analysis was performed within four hours post
venepuncture, after which the specimen was centrifuged, plasma
separated and stored in 1.5 ml Eppendorf® vials at −70°C for CRP
analysis.   Clotted specimens were centrifuged; serum separated and
stored in 1.5 ml Eppendorf® vials at −70°C.   Platelet free plasma was
obtained by centrifuging citrated blood twice, which was aliquoted and
stored in 1.5 ml Eppendorf® vials at −70°C.

3.3.2 Fibrin network architecture variables

These variables were performed on citrate plasma. Thirty-five micro-litres
of 10.000 KIU/ml Trasylol®/aprotinin per nine volumes of citrate plasma
was added, and thus used as an inhibitor of fibrin(ogen) lysis. Plasma with
trasylol was used for analysis of fibrin network content, compaction and
mass-length ratio from turbidity. Permeability was not performed because
of the great degree of variation and the complexity of the method.
Network lysis rate was performed on plasma without Trasylol®.

3.3.2.1 Network fibrin content

The method of Ratnoff & Menzies (1951) was used for duplicate
determination of all network fibrin content. For network fibrin content

0.9 ml plasma was pipetted into test tubes containing one gram glass beads and was clotted by addition of 100 µL Thrombin Reagent (1 IU/ml Thrombin final concentration, 25 mM Ca^{++} final concentration). Samples were left overnight for maximum polymerisation. All samples were centrifuged at 1300 X g and the supernatants were discarded. The isolated networks were washed three times with saline solution. One ml 2.5 M NaOH was dispensed into each test tube and the networks were dissolved by heating the tubes for 15 minutes at 95℃. The samples were left to cool at room temperature. Seven millilitres water and three ml 1.9 M sodium carbonate were dispensed into each tube, containing 200 µL of the NaOH-fibrin suspension. The tubes were vortexed and mixed with one ml Folin-Ciocalteu's phenol reagent. Samples were incubated at room temperature for 20 minutes and the absorbance measured at 650 nm. Different concentrations of albumin were dissolved in 2.5 M NaOH and used to prepare a standard curve. This standard curve was used to calculate the concentration of fibrin present in unknown samples.

3.3.2.2 Mass-length ratio from turbidity (µT)

The mass-length ratio from turbidity (µT) was determined in triplicate for each sample, using the method as described by Nair *et al.* (1991). Nine hundred µL of platelet-poor plasma was pipetted into micro-cuvettes of one centimetre path length. The plasma was mixed and clotted by

addition of 100 μL Thrombin Reagent (1 IU/ml Thrombin final concentration, 25 mM Ca^{++} final concentration). Samples were left overnight for maximum polymerisation.

The intercepts, A, in plots of $c/T(\lambda)^3$ as a function of $1/(\lambda)^2$ were used to calculate μT according to the equation :

$$\mu T = [10/1.48xA] \times 10^{12} \text{ daltons/cm}$$

with c the network content, T the absorbance $\times$ 2.304 and λ the wavelength. Turbidity (optical density) was measured at a range of wavelengths between 600 and 800 nm.

3.3.2.3 Compaction of fibrin networks

Compaction was measured in duplicate using the method as described by Dhall *et al.* (1976). Nine hundred micro-litre of 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 (1 IU/ml Thrombin final concentration, 25 mM Ca^{++} final concentration). Samples were left overnight for maximum polymerisation. After centrifugation at 8000 X g for 45 seconds the volume of expelled sample from the fibrin networks was determined using a one ml syringe and expressed as a percentage of the initial volume.

3.3.2.4 <u>Fibrin network lysis rate</u>

The network lysis rate was measured in duplicate using a method developed at the Fibrinogen Unit, Research laboratory. Ninety µL of plasma was mixed and clotted by the introduction of 10 µL thrombin reagent (1 IU/ml thrombin final concentration, 25 mM calcium final concentration) in microtiter plates. After total polymerisation took place, 50 µL of streptokinase with a final concentration of 100 U/ml was introduced to start lysis of networks. The absorbance was measured with an ELISA plate reader at 608 nm for six hours at 10 minute intervals. The lysis rate was determined by plotting the time versus change in absorbance.

3.3.3 Coagulation factor determinations

All the coagulation measurements were performed on the Berhring coagulation timer automatic analyser, using methods listed in Table 3.4.

TABLE 3.4 Methods used for coagulation factor determinations

Measured variable	Catalogue no	Brand name	Principle
Factor VII	OUHP 17	Thromborel® S	Clot formation
Factor VIII	OQGS 17	Pathromtin® SL	Clot formation
Antithrombin III	OWWR 17	Berichrom® AT III	Chromogenic
Fibrinogen	OWZG 15	Multifibren® U	Clauss' method
Fibrin monomers	OWXZ 11	Berichrom® FM	Chromogenic
D-Dimers$^{\$}$	OQWW 11	D-Dimer Plus	Turbimetric

$^{\$}$*D-Dimer analyses were performed only on samples drawn on day 8 and 36. These reagents where all provided by Dade Berhring, Marburg, Germany*

3.3.4 Chemical analyses

All the chemical analyses were performed on the Hitachi 902, automatic analyser, using methods listed in Table 3.5.

TABLE 3.5 Methods used for chemical variables determination

Variables	Cat. No.	Principle	Supplier
Total cholesterol	1489323	Enzymatic colorimetric	Roche Diagnostics
LDL-Cholesterol	1985604	Enzymatic colorimetric	Roche Diagnostics
HDL-Cholesterol	1930672	Enzymatic colorimetric	Roche Diagnostics
Triglycerides	1488872	Enzymatic colorimetric	Roche Diagnostics
Free fatty acids	FA115	Enzymatic colorimetric	Randox Laboratories Ltd

3.3.5 Full blood counts and C–Reactive protein measurements

Full blood count was performed on the Coulter$^{®}$ MicroDiff 18, cell counter. The C-reactive protein (CRP) was determined on the MICROS CRP analyser, with a turbidimetric method where anti-CRP antibody was coupled with latex particles.

3.4 STATISTICAL ANALYSES

The statistical analyses were performed by the Department of Biostatistics, University of the Free State, Bloemfontein, using the SAS$^{®}$ programme. Data were summarised as means and standard deviation for each measured variable. The differences between the means of the same variable but of a different time period were compared using the Student t-test. The difference between the different groups was compared using the ANOVA.

CHAPTER 4

RESULTS

4.1 INTRODUCTION

Dietary fibre is said to help in the prevention of westernised diseases but the exact mechanism of action is not known. In chapter two it has been explained that, dietary fibre is fermented in the large gut to short-chain fatty acids (acetate, propionate and butyrate). It was also explained that, it is hypothesised that these SCFAs may, in part, contribute in some way to the beneficial effects of dietary fibre intake on human metabolism. The aim of this study was to test the effects of different combinations of short-chain fatty acids on some metabolic risk markers generally associated with the development of cardiovascular heart disease in westernised African men.

A summary of the baseline results for the entire study group will be presented first, followed by the results of the different supplementation groups. The results of the different groups were sub-divided into three categories, namely; (i) general health profile, which includes the anthropometrical, full blood count and CRP data; (ii) haemostatic profile, and (iii) lipid profile. However, this study highlights the effects of the given

supplements on haemostatic risk factors and only a short description of the other risk variables will be given.

4.2 BASELINE RESULTS OF THE ENTIRE STUDY GROUP

Two baseline measurements were taken from each volunteer separated by a time-interval of seven days. All subjects consumed placebo supplements for standardisation purposes between the two baseline visits. The results of the two baseline measurements will now be presented in order to provide information regarding the more general characteristics of the study population as a whole. The reference ranges of the different variables are included as an aid to judging the results.

4.2.1 General health profile of the entire study group for both baseline visits

Anthropometrical measurements, blood cell counts and CRP concentrations of the two baseline visits for most of the study group were within the normal reference range, except for a few individual outliers. However, none of the outliers were clinically abnormal. All subjects were therefore included in the study group.

The results of baseline one was comparable to those of baseline two, with no statistically significant differences between the two visits.

Table 4.1   Anthropometrical, blood cell counts and CRP variables of the group as a whole

VARIABLE	Normal Range	Baseline 1				Baseline 2			
		X	SD	Min	Max	X	SD	Min	Max
Length (cm)		170.0	6.5	157.0	189.0				
Weight (cm)		66.3	8.9	51.4	87.5	67.2	8.9	52.3	86.9
BMI (kg/m^2)		22.8	2.3	29.0	17.3	23.0	2.4	18.0	29.5
Waist (cm)		77.2	6.9	66.0	94.0	77.7	7.1	66.0	94.0
Hip (cm)		95.4	5.9	74.0	110.0	95.9	6.2	74.0	110.0
SBP (mmHg)	125±13.6[a]	118	11	90	160	118	12	91	162
DBP (mmHg)	78±9.9 [a]	81	16	60	130	83	12	60	130
WBC (x10^3/µL)	2.5-8.5[b]	5.95	2.05	3.20	12.00	5.66	1.51	2.90	9.00
RBC (x10^3/µL)	4.5-5.9 [b]	5.16	0.53	4.31	6.77	5.17	0.57	4.25	6.93
Hb (g/dL)	13.7-17.8 [b]	15.9	1.1	13.0	18.1	15.8	1.5	12.8	22.6
Haematocrit (%)	41-52 [b]	45.8	4.1	37.9	60.2	45.2	4.1	35.7	61.0
PLT (x10^3/µL)	150-400 [b]	281	56	182	396	297	67	149	498
CRP (mg/dL)	0 - 0.3[c]	0.22	0.29	0.01	1.35	0.27	0.33	0.01	1.91

(X = mean; SD = standard deviation; BMI = Body Mass Index; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; WBC = White Blood Cell Count; RBC = Red Blood Cell Count; CRP = C-reactive protein; Hb = Haemoglobin; PLT = Platelet count).
a = Solomon, Schmidt & Adragna, 1990; **b =** Department Haematology and Cell Biology, University of the Free State & NHLS, 2005; c = Department of Chemical Pathology, University of the Free State & NHLS, 2005.

4.2.2 Haemostatic profile of the entire study group for both baseline visits

Haemostatic measurements of the two baseline visits for the group as a whole were within the normal reference range. The results of baseline one was comparable to those of baseline two, with no statistically significant differences between the two visits.

Table 4.2 Mean haemostatic variables of the study group as a whole

VARIABLE	Normal Range	Baseline 1				Baseline 2			
		X	SD	Max	Min	X	SD	Max	Min
FVII (%)	50-150 [b]	101.4	8.2	121.3	82.0	102.5	9.8	121.5	85.0
FVIII (%)	50-150 [b]	88.1	8.1	108.0	72.8	92.4	11.2	116.0	75.8
Fbg (g/L)	2.0-4.0 [b]	2.77	0.91	4.93	1.80	3.28	1.26	6.72	1.56
ATIII (%)	76-122 [b]	112.6	11.7	132.4	78.3	112.6	13.3	132.8	82.9
DD (µg/dL)	<500 [b]					97.6	69.9	271.0	26.0
FM (mg/L)		13.6	1.8	19.2	10.5	13.7	2.4	20.5	9.2
Comp (%)		16	6	26	8	14	5	23	7
µT (x10^{12} Da/cm)		11.8	6.3	32.3	2.7	7.2	5.6	28.1	2.0
NFC (g/L)		2.0	0.5	1.0	3.8	2.9	1.3	5.8	1.0

(X = mean; SD = standard deviation; FVII = blood clotting factor VII; FVIII = blood clotting factor FVIII; ATIII= antithrombin III; FM = Fibrin Monomer; µT = mass/length-ratio of fibrin strands in fibrin networks; Fbg = Fibrinogen; Comp = Compaction; NFC = Fibrin Content of Fibrin Networks)

b = Department Haematology and Cell Biology, University of the Free State & NHLS, 2005.

A graphic presentation of the changes that occur during fibrin network lysis, as measured by means of turbidimetry, for both baseline visits of the group as a whole follow more or less the same pattern. Similar rates of network lysis where measured up until 10 minutes, after which the curves both plateau at 180 minutes. However, in comparison with baseline one, baseline two plateaus at a much higher absorbance.

Network lysis rate: Entire study Group
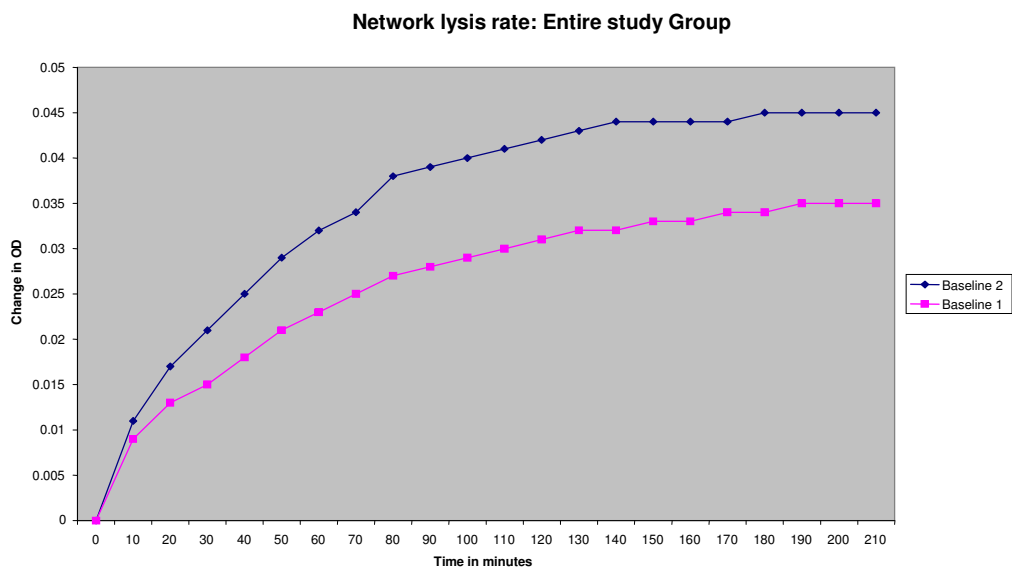
Figure 4.1 A turbidimetric presentation of the mean fibrin network lysis rate of the entire study group at baseline one and two

4.2.3 Lipid profile of the entire study group for both baseline visits

Mean lipid measurements of the two baseline visits for the entire group were within the normal reference range. The outliers were not clinically abnormal, and therefore not excluded from the study. The results of baseline one was comparable to those of baseline two, with no statistically significant differences between the two visits.

Table 4.3   Mean blood lipid concentrations of the study group as a whole

VARIABLE	Normal Range	Baseline 1				Baseline 2			
		X	SD	Max	Min	X	SD	Max	Min
TC	2.10-5.32	4.36	0.88	6.3	2.3	4.46	0.84	7.2	2.6
TG	0.30-1.70 [c]	1.17	0.50	2.21	0.50	1.17	0.61	2.72	0.53
HDL-C	0.75-1.9 [c]	1.41	0.47	3.6	0.7	1.24	0.28	2.0	0.7
LDL-C	2.24-4.6 [c]	2.35	0.83	4.51	0.8	2.67	0.78	3.64	1.24
% HDL-C		33.7	9.5	65.5	16.4	28.5	7.3	47.1	14.8
NEFA		4.78	2.74	9.10	1.40	3.68	1.70	9.10	1.20

(X = mean; SD = standard deviation; TC = serum-Total Cholesterol; TG = serum-Triglycerides; HDL-C = serum-High-Density Lipoprotein Cholesterol; LDL-C = serum-Low-Density Lipoprotein Cholesterol; %HDL-C = HDL-C/TC * 100; NEFA = Non-Esterified Fatty Acids, The measurement unit for all the lipid variables was mmol/L except for % HDL-C which was percentage)

[c] = Department of Chemical Pathology, University of the Free State & NHLS, 2005.

4.3   RESULTS OF THE INTERVENTION STUDY

This section will be used to present the results measured in metabolic risk markers after supplementation with the three different short-chain fatty acid supplements.   Results for short-term changes (after 22 days of supplementation), end of supplementation changes (after 36 days of supplementation) and a wash out period of one week (after 43 days of supplementation) will be reported.   Focus will mainly be on the differences

between day 8 (baseline 2; start of supplementation) and day 36 (end of supplementation).

4.3.1 The Placebo Supplement Intervention Study Results

The group consumed a placebo for the entire study period. This group was composed of 25 volunteers but only 21 volunteers' results were used. The other four subjects' results were excluded because some of the visits' haemostatic specimens were clotted.

4.3.1.1 <u>Mean changes in the general health profile of the placebo supplement study group</u>

No significant changes were measured in any of the general health indicators from day 8 (start of supplementation phase) to day 36 (end of supplementation phase) within the placebo supplement group.

Table 4.4 Mean changes in anthropometric variables of the placebo supplement study group

VARIABLE		Day 1	Day 8	Day 22	Day 36	Day 43
Length (cm)	X	171.0				
	SD	6.6				
Weight (kg)	X	69.0	70.0	70.3	69.8	70.0
	SD	10.6	10.2	11.3	10.4	10.5
BMI (kg/m^2)	X	23.5	23.8	24.0	23.8	23.9
	SD	3.1	3.0	3.2	2.9	3.0
Waist (cm)	X	79.8	80.7	79.1	79.11	79.1
	SD	8.6	8.5	8.1	8.3	8.0
Hip (cm)	X	96.2	96.8	97.9	97.5	98.2
	SD	8.4	8.7	6.3	6.2	6.7
SBP (mmHg)	X	120	124	122	116	116
	SD	14	14	13	11	10
DBP (mmHg)	X	85	87	85	83	83
	SD	15	15	15	10	11

(X = mean; SD = standard deviation; BMI = Body Mass Index; SBP = Systolic Blood Pressure;

DBP = Diastolic Blood Pressure)

Table 4.5 Mean changes in the full blood count and CRP variables of the placebo supplement study group

VARIABLE		Day 1	Day 8	Day 22	Day 36	Day 43
WBC ($x10^3/\mu L$)	X	6.24	5.91	5.27	5.29	5.82
	SD	2.06	1.62	1.52	1.24	1.79
RBC ($x10^3/\mu L$)	X	5.25	5.10	5.00	5.00	5.19
	SD	0.44	0.50	0.52	0.42	0.62
Haemoglobin (g/dL)	X	16.1	15.3	15.2	15.6	15.8
	SD	1.1	1.0	1.3	1.2	1.2
Haematocrit (%)	X	46.4	44.2	43.3	43.6	44.9
	SD	3.6	3.0	3.1	3.2	3.6
Platelets ($x10^3/\mu L$)	X	307	304	296	300	348
	SD	58	66	61	56	52
CRP (mg/dL)	X	0.17	0.24	0.21	0.11	0.16
	SD	0.17	0.15	0.14	0.12	0.23

(X = mean; SD = standard deviation; WBC = White Blood Cell Count; RBC = Red Blood Cell Count; CRP = C-reactive protein)

4.3.1.2 Mean changes in the haemostatic profile of the placebo supplement study group

A statistically significant decrease (p = 0.047) in plasma fibrinogen concentration was measured from the start of supplementation (day 8) to the end of supplementation (day 36) within the placebo supplemented group. No other significant changes in any of the other haemostatic variables were measured.

Table 4.6 Mean changes in the haemostatic variables of the placebo supplement study group

VARIABLE		Day 1	Day 8	Day 22	Day 36	Day 43
FVII (%)	X	102.9	103..5	98.51	99.6	98.8
	SD	9.4	7.8	12.3	7.3	9.5
FVIII (%)	X	88.5	93.6	87.2	88.50	90.0
	SD	9.5	10.0	8.1	6.6	9.6
Fibrinogen (g/L)	X	2.80	3.39*	2.80	2.79*	2.90
	SD	0.88	1.17	0.71	0.76	1.24
Albumin/ Fibrinogen	X	17.2	15.5	16.8	16.8	19.2
	SD	4.7	5.2	4.9	4.1	7.2
ATIII (%)	X	113.7	114.4	109.1	107.7	109.5
	SD	9.9	10.0	9.3	10.9	10.2
D-Dimer (μg/dL)	X		89.94		138.68	
	SD		64.17		126	
FM (mg/L)	X	13.2	13.4	12.3	12.2	12.4
	SD	1.4	3.0	2.5	3.1	3.7
Compaction (%)	X	17.7	13.6	15.1	14.3	14.5
	SD	6.7	6.1	4.8	3.7	5.1
µT ($x10^{12}$ Da/cm)	X	11.8	7.4	5.5	5.0	5.8
	SD	5.6	5.1	2.5	2.0	2.9
NFC (g/L)	X	2.6	2.9	2.0	2.7	2.6
	SD	0.9	1.4	0.6	1.6	1.9

(X = mean; SD = standard deviation; FVII = blood clotting factor VII; FVIII = blood clotting factor FVIII; ATIII= antithrombin III; CRP = C-reactive Protein; FM = Fibrin Monomer; µT = mass/length-ratio of fibrin strands in fibrin networks; NFC = Fibrin Content of Fibrin Networks; * = differs significantly with $p<0.05$; ** = differs significantly with $p<0.01$)

A graphic presentation of the turbidimetric changes that occur during fibrin network lysis, for all visits for the placebo supplemented group, followed more or less the same pattern. Similar rates of lysis where initially measured up until 20 minutes, after which the graphs all reached a plateau at 190 minutes. However, in comparison to all the other study periods, day 22 (after 2 weeks of experimental placebo supplementation) reached the plateau phase at a much lower absorbance.

Network lysis rate: Placebo Supplement Study Group

Figure 4.2 A turbidimetric presentation of the mean fibrin network lysis rates of the placebo group for the entire study period

4.3.1.3 <u>Mean changes in the lipid profile of the placebo supplement study group</u>

No significant changes in any of the lipid variables were measured between day 8 (start of supplementation) and day 36 (end of supplementation) within the placebo supplemented study group.

Table 4.7   Mean changes in the lipid variables of the placebo supplement study group

VARIABLE		Day 1	Day 8	Day 22	Day 36	Day 43
TC	X	4.21	4.31	4.29	4.32	4.43
(mmol/L)	SD	0.95	0.70	0.97	0.47	0.89
TG	X	1.26	1.30	1.12	1.27	1.18
(mmol/L)	SD	0.40	0.84	0.47	0.41	0.75
HDL-C	X	1.22	1.18	1.34	1.26	1.44
(mmol/L)	SD	0.27	0.27	0.27	0.25	0.31
LDL-C	X	2.27	2.47	2.42	2.47	2.55
(mmol/L)	SD	0.73	0.65	0.76	0.50	0.79
%HDL-C	X	31.3	28.1	32.3	29.6	32.9
(%)	SD	7.1	7.8	7.1	6.5	8.8
NEFA	X	4.99	3.83	3.68	3.59	3.66
(mmol/L)	SD	1.94	1.62	1.99	2.16	2.44

(X = mean; SD = standard deviation; TC = serum-Total Cholesterol; TG = serum-Triglycerides;

HDL-C = serum-High-Density Lipoprotein Cholesterol; LDL-C = serum-Low-Density Lipoprotein

Cholesterol; %HDL-C = HDL-C/TC * 100; NEFA = Non-Esterified Fatty Acids)

4.3.2 The Acetate-Propionate (50/50) Supplement Intervention Study Results

This study group consisted of 25 volunteers that consumed placebo supplement for a period of one week, followed by four weeks of experimental supplementation, which was composed of 50% acetate and 50% propionate salts (sodium, potassium and calcium), after which one week of placebo supplementation followed. Only 23 volunteers' results were used in the final calculation because the others' haemostatic specimen were clotted and therefore excluded.

4.3.2.1 <u>Mean changes in the general health profile of the acetate-propionate supplement study group</u>

No significant changes were measured in any of the general health indicators between day 8 (start of supplementation) and day 36 (end of supplementation) for the acetate-propionate supplement group.

Table 4.8   Mean changes in anthropometric variables of the acetate-propionate (50%-50%) supplement study group

VARIABLE		Day 1	Day 8	Day 22	Day 36	Day 43
Length (cm)	X	170.0				
	SD	6.2				
Weight (kg)	X	65.1	66.1	65.3	65.7	66.6
	SD	7.4	8.2	8.1	7.8	7.5
BMI (kg/m^2)	X	22.6	22.9	22.8	22.8	23.0
	SD	1.9	2.1	2.1	2.1	1.9
Waist (cm)	X	75.5	76.4	75.9	76.5	75.6
	SD	5.2	5.1	5.5	4.9	4.8
Hip (cm)	X	95.5	96.5	96.2	96.4	96.0
	SD	4.3	5.1	5.1	4.7	4.8
SBP (mmHg)	X	120	116	117	116	118
	SD	10	12	7	8	8
DBP (mmHg)	X	81	81	79	79	83
	SD	9	9	7	8	9

(X = mean; SD = standard deviation; BMI = Body Mass Index; SBP = Systolic Blood Pressure;

DBP = Diastolic Blood Pressure)

Table 4.9 Mean changes in the full blood count and CRP concentrations of the

acetate-propionate (50%-50%) supplement study group

VARIABLE		Day 1	Day 8	Day 22	Day 36	Day 43
WBC	**X**	5.96	5.78	5.89	5.88	5.65
(x10³/µL)	**SD**	2.47	1.42	1.30	1.63	1.43
RBC	**X**	5.33	5.32	5.17	5.33	5.23
(x10³/µL)	**SD**	0.65	0.63	0.49	0.54	0.56
Haemoglobin	**X**	16.2	16.1	15.5	15.7	15.6
(g/dL)	**SD**	1.2	1.9	1.3	1.2	1.5
Haematocrit	**X**	47.3	46.1	44.4	45.6	44.8
(%)	**SD**	5.0	5.4	4.3	4.4	4.5
Platelets	**X**	264	301	282	331	340
(x10³/µL)	**SD**	50	79	85	83	90
CRP	**X**	0.23	0.25	0.28	0.27	0.15
(mg/dL)	**SD**	0.29	0.28	0.24	0.49	0.18

(X = mean; SD = standard deviation; WBC = White Blood Cell Count; RBC = Red Blood Cell

Count; CRP = C-reactive protein)

4.3.2.2 <u>Mean changes in the haemostatic profile of the acetate-propionate supplement study group</u>

A statistically significant decrease in plasma factor VIII (p=0.016), antithrombin III (p<0.001), and an increase in plasma fibrinogen concentration (p=0.048) and the albumin/fibrinogen ratio (p=0.044), was measured between day 8 and day 36 within the acetate-propionate supplementation group. No other changes in any of the other haemostatic variables were significant.

Table 4.10 Mean changes in the haemostatic variables of the acetate-propionate (50%-50%) supplement study group

VARIABLE		Day 1	Day 8	Day 22	Day 36	Day 43
FVII (%)	X	101.0	101.6	99.1	97.2	97.8
	SD	7.6	7.0	7.0	6.5	7.9
FVIII (%)	X	89.3	91.12*	89.7	90.9*	88.8
	SD	6.4	11.2	11.1	8.3	7.6
Fibrinogen (g/L)	X	2.90	2.98*	2.58	3.04*	2.69
	SD	1.15	0.96	0.61	0.89	0.70
Albumin/ Fibrinogen	X	17.8	17.4*	17.9	15.7*	18.6
	SD	5.7	5.9	3.9	4.7	5.5
ATIII (%)	X	113.2	114.3**	107.1	108.4**	109.1
	SD	12.7	13.1	7.4	9.5	7.7
D-Dimer (μg/Dl)	X		118.56		121.20	
	SD		89.31		75.70	
FM (mg/L)	X	13.5	13.7	12.4	12.0	12.6
	SD	1.3	2.1	2.3	2.2	2.6
Compaction (%)	X	16.1	14.3	14.9	14.1	15.0
	SD	5.8	4.7	3.8	3.6	3.7
μT (x10^{12} Da/cm)	X	13.0	8.5	8.0	6.7	5.7
	SD	7.4	7.5	5.7	2.3	3.3
NFC (g/L)	X	1.8	3.0	2.5	2.6	2.7
	SD	0.6	1.1	0.8	1.3	1.5

(X = mean; SD = standard deviation; FVII = blood clotting factor VII; FVIII = blood clotting factor FVIII; ATIII= antithrombin III; CRP = C-reactive Protein; FM = Fibrin Monomer; μT = mass/length-ratio of fibrin strands in fibrin networks; NFC = Fibrin Content of Fibrin Networks; * = differs significantly with $p < 0.05$; ** = differs significantly with $p < 0.01$)

A turbidimetric presentation of the changes in the network lysis rate of all the study periods, for the acetate-propionate study group, showed a sharp rise in optical density for the first 40 minutes and reaching a plateau phase at approximately 190 minutes, except for day 22, where the maximum plateau phase was reached at 240 minutes. In comparison with the other study periods day 1 reached a much lower maximum absorbance, while day 43 reached the highest maximum absorbance.

Network lysis rate: Acetate-Propionate Study Group
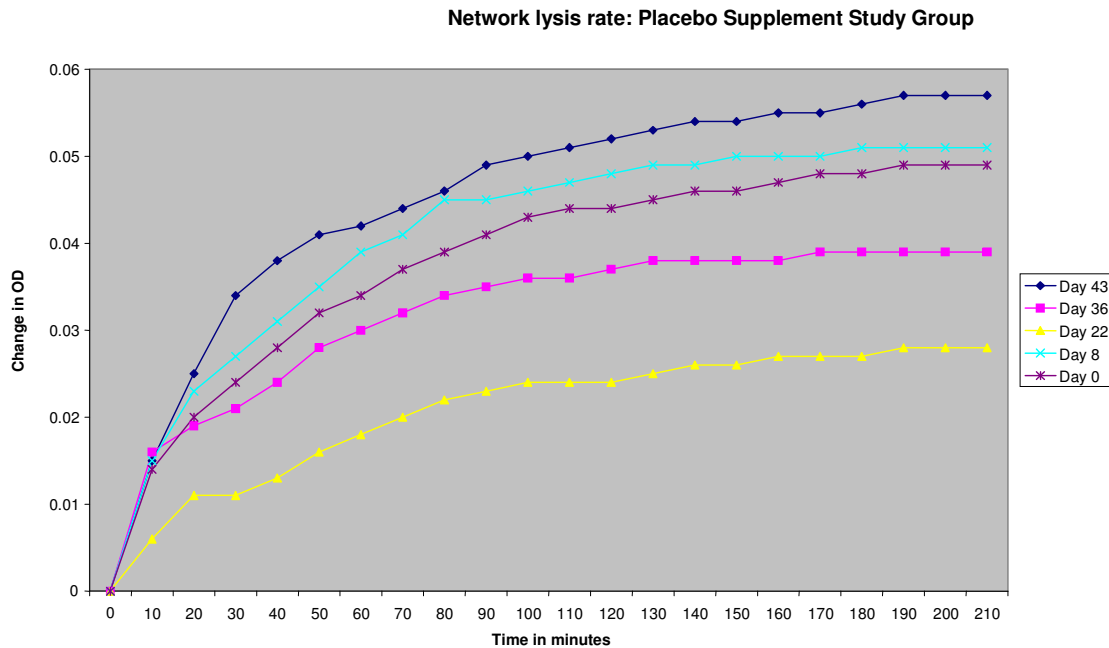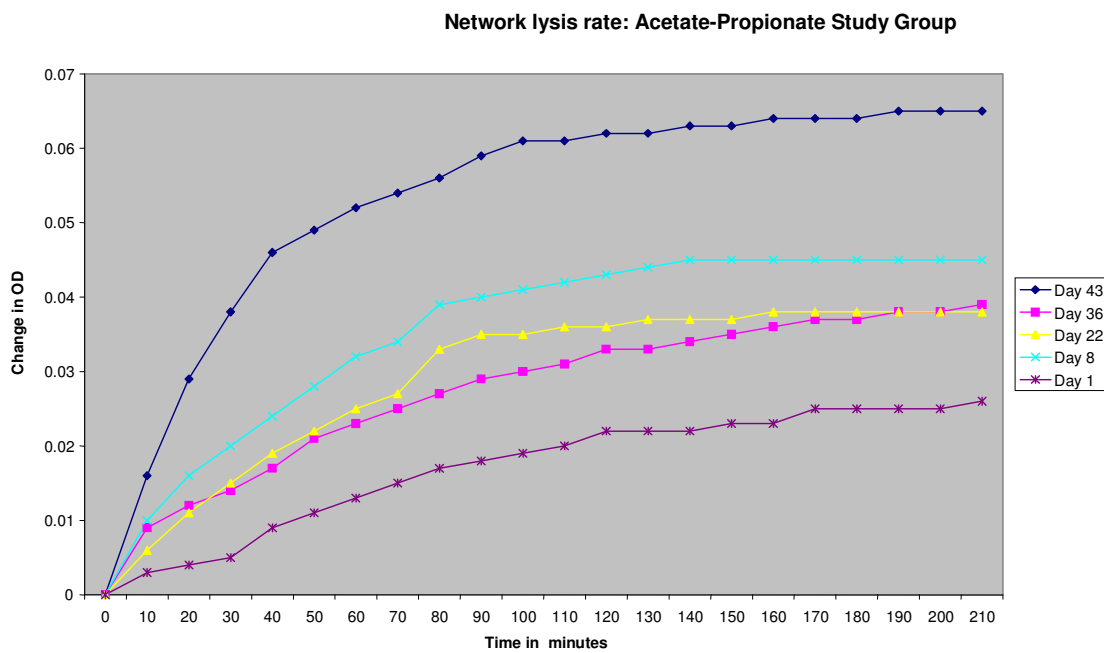
Figure 4.3 A turbidimetric presentation of the mean fibrin network lysis rate of the acetate-propionate group for the entire study period

4.3.2.3 <u>Mean changes in the lipid profile of the acetate-propionate supplement study group</u>

A statistically significant increase in both serum triglyceride ($p < 0.001$) and percentage HDL-C ($p = 0.017$) were found between day 8 and day 36 within the acetate-propionate supplement group. LDL-C ($p < 0.001$) decreased significantly in that same period. No other measured changes in any of the other lipid variables were significant.

Table 4.11 Mean changes in the lipid variables of the acetate-propionate (50%-50%) supplement study group

VARIABLE		Day 1	Day 8	Day 22	Day 36	Day 43
TC	X	4.47	4.85	4.96	4.58	4.74
(mmol/L)	SD	0.75	0.82	1.24	0.83	0.91
TG	X	1.17	1.16**	1.08	1.26**	1.04
(mmol/L)	SD	0.67	0.53	0.31	0.56	0.51
HDL-C	X	1.56	1.27	1.51	1.36	1.52
(mmol/L)	SD	0.65	0.28	0.50	0.49	0.54
LDL-C	X	2.39	3.10**	3.06	2.64**	2.78
(mmol/L)	SD	0.85	0.79	1.14	0.73	0.83
%HDL-C	X	34.9	26.3*	30.2	30.2*	31.8
(%)	SD	11.9	6.5	6.9	9.3	8.3
NEFA	X	3.9	3.5	3.8	2.9	3.1
(mmol/L)	SD	2.1	1.9	2.2	1.5	1.7

(X = mean; SD = standard deviation; TC = serum-Total Cholesterol; TG = serum-Triglycerides; HDL-C = serum-High-Density Lipoprotein Cholesterol; LDL-C = serum-Low-Density Lipoprotein Cholesterol; %HDL-C = HDL-C/TC * 100; NEFA = Non-Esterified Fatty Acids; * = differs significantly with $p < 0.05$; ** = differs significantly with $p < 0.01$)

4.3.3 The High-Acetate Supplement Intervention Study Results

The baseline results of this group were comparable to the results of the other two groups. The results presented are of 22 of the 25 volunteers, the other three's haemostatic specimen were clotted. The volunteers also consumed placebo supplements for a period of at least one week, followed by four weeks of supplementation, which was composed of 70% acetate, 15% propionate and 15% butyrate, and ending with a week of placebo.

4.3.3.1 <u>Mean changes in the general health profile of the high-acetate supplement study group</u>

No significant changes were measured in any of the general health indicators between day 8 (start of supplementation) and day 36 (end of supplementation) for the high acetate supplement group.

Table 4.12 Mean changes in the anthropometrical variables of the high-acetate supplement group

VARIABLE		Day 1	Day 8	Day 22	Day 36	Day 43
Length (cm)	X	170.6				
	SD	7.2				
Weight (kg)	X	65.0	65.6	66.6	65.9	66.9
	SD	8.2	8.3	8.2	8.6	7.5
BMI (kg/m^2)	X	22.3	22.5	22.6	22.6	22.7
	SD	1.8	1.8	1.9	1.8	1.5
Waist (cm)	X	76.4	76.1	75.6	75.7	77.4
	SD	5.9	6.7	8.5	6.6	6.3
Hip (cm)	X	94.6	94.2	93.9	94.1	95.2
	SD	4.4	4.0	8.0	4.3	4.5
SBP (mmHg)	X	115	115	118	116	116
	SD	7	8	9	8	6
DBP (mmHg)	X	81	80	80	82	81
	SD	8	8	10	7	7

(X = mean; SD = standard deviation; BMI = Body Mass Index; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure)

Table 4.13 Mean changes in the full blood count variables of high-acetate supplement group

VARIABLE		Day 1	Day 8	Day 22	Day 36	Day 43
WBC $(x10^3/\mu L)$	X	5.68	5.27	6.08	5.09	5.34
	SD	1.61	1.6	2.17	1.70	1.51
RBC $(x10^3/\mu L)$	X	4.90	5.05	5.10	5.25	5.19
	SD	0.39	0.58	0.52	0.60	0.82
Haemoglobin (g/dL)	X	15.5	16.0	16.1	16.1	15.9
	SD	0.9	1.3	1.6	1.3	1.8
Haematocrit (%)	X	43.7	45.1	45.0	46.0	45.9
	SD	2.7	4.4	4.0	4.0	4.6
Platelets $(x10^3/\mu L)$	X	273	285	299	297	298
	SD	53	60	45	67	81
CRP (mg/dL)	X	0.28	0.32	0.21	0.24	0.21
	SD	0.42	0.50	0.23	0.36	0.28

(X = mean; SD = standard deviation; WBC = White Blood Cell Count; RBC = Red Blood Cell Count; CRP = C-reactive protein)

4.3.3.2 <u>Mean changes in the haemostatic profile of the high-acetate supplement study group</u>

A statistically significant decrease in plasma factor VII (p=0.018), factor VIII (p=0.011), antithrombin III (p<0.001) and fibrin monomers (p=0.002) was measured between day 8 and day 36 within the high acetate supplementation group, while compaction showed an increase (p=0.008). No other changes in any of the other haemostatic variables were significant.

Table 4.14 Mean changes in the haemostatic variables of the high-acetate supplement group

VARIABLE		Day 1	Day 8	Day 22	Day 36	Day 43
FVII (%)	X	100.5	102.5*	101.3	101.1*	99.3
	SD	8.0	13.7	7.00	6.4	7.6
FVIII (%)	X	86.1	92.6*	89.6	87.6*	86.3
	SD	8.4	12.8	10.3	6.0	5.7
Fibrinogen (g/L)	X	2.59	3.48	3.16	2.80	2.71
	SD	0.57	1.61	1.20	0.84	0.84
Albumin/ Fibrinogen	X	18.0	15.2	16.3	16.9	16.2
	SD	3.8	6.4	4.3	4.7	5.0
ATIII (%)	X	110.6	109.2**	104.3	103.0**	105.2
	SD	12.4	16.0	8.6	9.9	12.5
D-Dimer (μg/dL)	X		82.53		111.76	
	SD		46.41		74.98	
FM (mg/L)	X	14.3	13.9**	12.7	12.1**	12.9
	SD	2.6	2.2	2.7	3.6	3.9
Compaction (%)	X	14.4	14.2**	14.1	13.7**	13.7
	SD	5.2	4.6	4.3	4.0	5.3
μT ($\times 10^{12}$ Da/cm)	X	10.4	5.9	8.7	7.2	5.9
	SD	5.5	3.7	5.1	2.6	2.2
NFC (g/L)	X	2.0	3.1	2.3	2.3	3.2
	SD	0.4	1.2	1.3	0.7	2.2

(X = mean; SD = standard deviation; FVII = blood clotting factor VII; FVIII = blood clotting factor FVIII; ATIII= antithrombin III; FM = Fibrin Monomer; μT = mass/length-ratio of fibrin strands in fibrin networks; NFC = Fibrin Content of Fibrin Networks; * = differs significantly with $p < 0.05$; ** = differs significantly with $p < 0.01$)

Figure 4.4 represents a graphic comparison between the changes in the fibrin network lysis rate during each study period, within the high-acetate supplement study group. Turbidimetric changes showed a sharp increase in absorbance for the first 80 minutes and reached the plateau phase at 180 minutes, except for day 22 which reached it by 240 minutes. In comparison to the others, day 43 reached a much higher absorbance. The rate of lysis of day 8 and day 36 did not differ significantly.
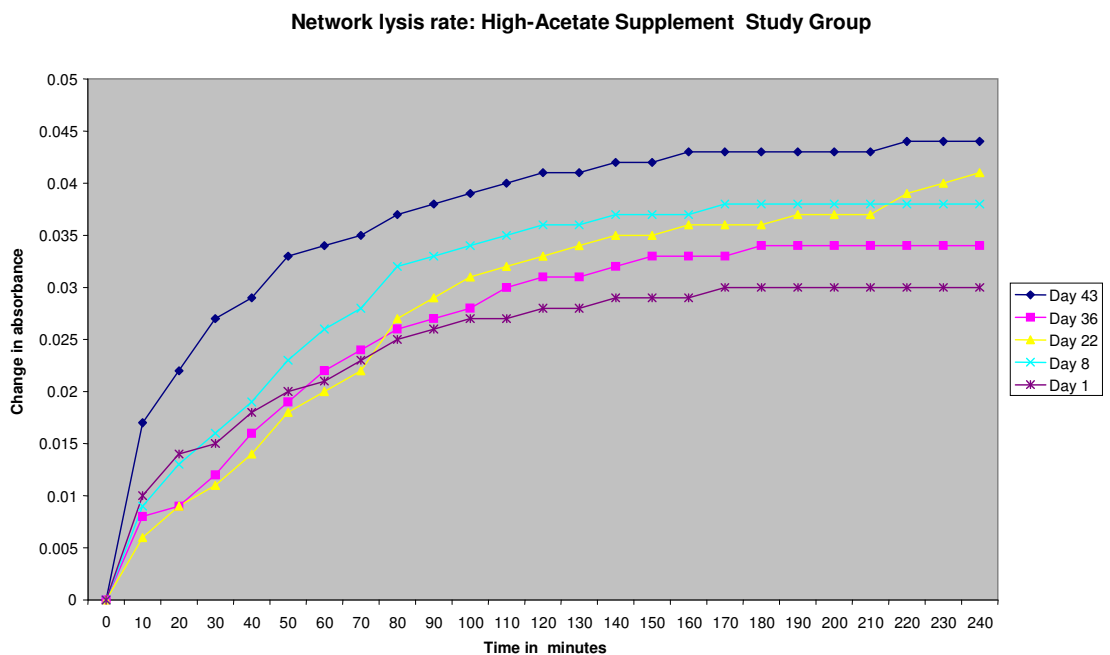
Figure 4.4 A turbidimetric presentation of the mean fibrin network lysis rate of the high acetate group for the entire study period

4.3.3.3 <u>Mean changes in the lipid profile of the high-acetate supplement study group</u>

A statistically significant increase in serum total cholesterol (p=0.001) was measured from day 8 to day 36 within the high acetate supplement group. No changes in any of the other lipid variables were significant.

Table 4.15 Mean changes in the lipid variables of the high-acetate supplement study group

VARIABLE		Day 1	Day 8	Day 22	Day 36	Day 43
TC	X	4.37	4.14**	4.71	4.32**	3.92
(mmol/L)	SD	0.99	0.85	0.91	0.82	0.80
TG	X	1.07	1.04	1.08	1.08	0.98
(mmol/L)	SD	0.41	0.41	0.39	0.37	0.28
HDL-C	X	1.44	1.28	1.41	1.35	1.37
(mmol/L)	SD	0.32	0.31	0.33	0.30	0.32
LDL-C	X	2.40	2.28	2.63	2.48	2.10
(mmol/L)	SD	0.92	0.63	0.81	0.74	0.72
%HDL-C	X	34.7	32.0	31.6	31.8	35.8
(%)	SD	8.7	7.2	7.7	6.8	8.7
NEFA	X	4.95	3.80	3.99	3.37	2.43
(mmol/L)	SD	2.28	1.65	2.55	1.71	1.18

(X = mean; SD = standard deviation; TC = serum-Total Cholesterol; TG = serum-Triglycerides; HDL-C = serum-High-Density Lipoprotein Cholesterol; LDL-C = serum-Low-Density Lipoprotein Cholesterol; %HDL-C = HDL-C/TC * 100; NEFA = Non-Esterified Fatty Acids; * = differs significantly with $p<0.05$; ** = differs significantly with $p<0.01$)

CHAPTER 5

DISCUSSION

5.1 INTRODUCTION

Coronary heart disease and stroke are considered as degenerative western diseases which in most cases can be prevented by a change in lifestyle, for example diet, physical activity and psychological well being. The link between haemostatic and lipid risk markers for coronary heart disease and stroke, and lifestyle (diet, exercise, stress, etc.) was explained in chapter two. In this study a group of westernised African male volunteers were recruited and fed two different combinations of short-chain fatty acids, as well as a placebo supplement, for a period of six weeks. Blood was drawn at different intervals and analysed to assess changes that were brought about by the different supplements. Focus will be mainly on the differences between start of supplementation (day 8) and end of supplementation (day 36). This chapter will be used to discuss the results which were presented in chapter four, therefore elucidating the effects that the different supplements had on haemostatic and lipid risk markers for coronary heart disease. The discussion of the baseline results of the entire study group will be presented first, followed by the intervention study.

5.2   BASELINE CHARACTERISTICS OF THE ENTIRE STUDY GROUP

Here a discussion of the baseline results is presented, so as to form a basis for understanding and interpreting the intervention study's results. The discussion of these baseline results will show if there were any abnormalities in this group of subjects, and will also evaluate the effect of placebo consumption for the run-in phase (which lasted a week), in so doing, its effectiveness in establishing a stable baseline.

5.2.1 General health profile of the entire study group at baseline

All subjects had blood pressures that were within the healthy range of the population group under investigation. The way in which the blood samples and other measurements were collected may have contributed to the 'healthy' results, mainly due to the fact that the subjects were all calm and treated with respect.   The nursing staff of the Defence Force was especially caring and friendly. No overweight or obese subjects were included for the purpose of this study, judging from the body-mass index, hip and waist measurements.   This could be ascribed to the inclusion criteria, and most importantly, the fact that the subjects were involved with physical activities most of the day, due to the nature of their occupation. The BMI and blood pressure measurements were similar to those obtained by epidemiological studies performed on Africans in South Africa; in Durban (Seedat, *et al.*, 1992), Cape Peninsula (Oelofse, *et al.*, 1996),

Free State (De Wet, 1999) and North West (James *et al.*, 2000). All these studies were investigating the effects of urbanisation on different risk factors for CHD and/or stroke.

Blood counts for the purpose of this study were mainly evaluated as means of 'marker' for the general health. It was not expected that the supplements should have any effect on these variables. However, it was important to know that anaemic subjects or subjects with low platelets or white cell counts, which could be indicative of a wide variety of different disease states, were not included. The blood cell counts reference ranges used in this study were established for African males in the Free State region (unpublished data – the experiments were done by the Department of Haematology and Cell Biology, University of the Free State, Bloemfontein, 1990), thus they are specific for the studied population. What can also be observed is that African people tend to have slightly lower red cell and white blood cell counts than the Caucasian population (Bain, 1995; Lewis, 2004). C-reactive protein (an acute phase protein) was measured to ensure that possible changes in other haematological parameters may not be due to inflammation or any other unknown acute phase response, and also because it is associated with CHD and CVD. Luizzo and his colleagues (1994) found elevated plasma CRP concentration in the majority of patients with unstable angina, myocardial infarction, and a history of unstable angina. While Ridker *et al*. (1997) suggested that the baseline plasma concentration of CRP in apparently

healthy men can predict the risk of first myocardial infarction and ischaemic stroke, independent of other risk factors.

There were no statistically significant changes in any of the general health indicators for both baseline one (day 1) and baseline two (day 2) of the entire study group. This indicates that the run-in phase with placebo managed to establish a stable baseline.

5.2.2 Haemostatic profile of the entire study group at baseline

Coagulation factor determination methods can be divided into two. In the first group, the factor activity is determined by clot formation time, which is proportional to the factor activity. The second group measures the factor directly, especially by immunological methods, usually indicated as factor concentration, it does not matter whether the factor is functional or not. The units of measurements in general use are percentage for factor activity, while for concentration µg/l or mmol/l is used (Hutton *et al.*, 1999). Thus it is important that when comparisons are made between studies that this difference is taken into account. In this study, factor activity was used as the preferred method since it measures only the functional factor.

Elevated FVII (Heinrich *et al.* 1994), FVIII (Pan *et al.* 1997) as well as plasma fibrinogen levels (Møller & Kristensen, 1991) are associated with increased risk of cardiovascular disease. Cut off values for plasma fibrinogen for the African population group are a point of argument. The main reason for this being the different views regarding the strength of

plasma fibrinogen as a predictor of stroke, compared to cardiovascular disease and the prevalence of these two diseases within the different population groups. The factor VII and VIII percentage activity were similar to those reported by De wet (1999). The fibrinogen concentration of this study group was slightly lower than that reported by James *et al.* (2000). Antithrombin III (ATIII) is considered a natural anticoagulant; its deficiency is associated with thrombosis (Walker, 2004). This might indicate that a higher than normal level will be to the advantage of the individual, but this needs thorough investigation and its association with coronary risk factor needs to be determined. Both fibrin monomer and d-dimer levels are considered as potential risk markers for development of cardiovascular events. The thrombin-catalysed released of the fibrinopeptides from fibrinogen results in the formation of a transient intermediate, termed fibrin monomer. Increased fibrin monomers levels are found in patients with hypercoagulable disease state, especially after a cardiovascular event. The fibrin monomer results reported in this study where higher than those reported by De Wet (1999). D-Dimer is a product of clotted fibrin breakdown, and is usually found in increased levels in patients with hypercoagulable disease states occurring before and after cardiovascular events. It also indicates that fibrinolysis has occurred, thus it is increased in disseminated intravascular coagulation (Laffan & Manning, 2001).

Fibrin network structure studies were performed because it is thought that the network structure contributes to the risk of developing coronary heart

disease (Mills *et al.*, 2002). The variables are mass-length ratio from turbidity (which measures fibre thickness), compaction (which measures the degree of cross-linking), and network lysis rate. The fibrin network content reported in this study was similar to those reported by Pieters *et al.* (2002) and Veldman *et al.* (1999), while the mass-length ratio was lower and the compaction higher in this study report. This discrepancy might be due to the different population groups that were used in these studies. Indicating that Africans might have these lower mass-length ratio and higher compaction, but this needs population studies to evaluate this supposition. Gabriel *et al.* (1992) has suggested that fibrin structure contributes to the regulation of the fibrinolytic rate; this is one of the reasons why fibrin network lysis rate studies were performed. The fibrin network lysis rates of the two baseline visits were similar, while the end-point absorbance of baseline one was lower. The reason for this observation was that baseline two's (day 8) plasma fibrinogen concentration and the network fibrin content was higher than that of baseline one (day 1). The fibrin network content is a measure of the amount of fibrin that is incorporated into the network structure, and it being high indicated that there was more fibrinogen was incorporated into the network structure. Therefore, more fibrinogen was available and indeed was incorporated into the network structure, thus resulting in a higher end point absorbance.

There were no statistically significant changes in any of the haemostatic variables for both baseline one (day 1) and baseline two (day 8) of the entire study group. This indicates that the run-in phase with placebo managed to establish a stable baseline.

5.2.3 Lipid profile of the entire study group at baseline

The lipid profile of this group of subjects is very interesting. The total cholesterol (TC) level of this group falls within the moderate risk category (4.10 – 5.50 mmol / L). The fasting TG levels fall below the high risk cut-off level of 2 mmol /L (Wilson *et al*., 1991). The HDL-C levels are relatively high when compared to the total cholesterol levels. However, in comparison with the HDL-C, concomitant high LDL-C levels (up to 54% of the TC) were measured. In general, this is very common within this population group. Consequently, at first glance, the lipid profiles seem healthy. However, the LDL-C levels (taking into consideration that it is lifestyle associated, as well as the group of the volunteers) place the subjects at risk of future cardiovascular events. The TC, TG, %HDL-C, HDL-C & LDL-C are comparable to those found by James, *et al.* (2000), Oelofse, *et al.* (1996) and Seedat, *et al.* (1992). The fasting Non-Esterified Fatty Acid level of this group falls within the accepted normal range. Normal daily values can fluctuate to as much as 10 mmol / L, depending on the fat content of the diet of an individual.

There were no statistically significant changes in any of the lipid variables for both baseline one (day 1) and baseline two (day 8) of the entire study group. This indicates that the run-in phase with placebo managed to establish a stable baseline.

5.3 INTERVENTION STUDY RESULTS

Here a discussion of results of different study groups is presented. Focus was on the results of day 8 (start of supplementation) and day 36 (end of supplementation). The baseline measurements of the different groups were comparable with no statistically significant differences thus any changes which were observed in any of the groups cannot be said that they were caused by different baseline results. From this observation it can be assumed that the changes were brought about by the different supplements.

5.3.1 The Placebo Supplement Intervention Study group

This study group consumed placebo supplements for the entire study period.

5.3.1.1 <u>Changes in the general health profile of the placebo supplement study group</u>

No statistically significant changes in any of the general health indicators within the placebo group were measured during the study, more importantly between day 8 (start of supplementation) and day 36 (end of supplementation). This can be seen as a positive result since the group consumed placebo and no significant changes were expected. It is interesting to note that the plasma CRP concentration of day 8 was higher than any of the other visits, though it was not statistically significant. This could be due to an unknown inflammatory response or infection.

5.3.1.2 <u>Changes in the haemostatic profile of the placebo supplement study group</u>

A statistically significant change was observed in the plasma fibrinogen concentration, which showed a decrease for the period from day 8 to day 36, and no statistically significant change was measured in any of the other haemostatic variables. When looking at all the visits it can be noticed that the fibrinogen concentration of day 8 was the highest for all of the visits, and this was also the case with factor VIII concentration though the change was not statistically significant. Since factor VIII, CRP and fibrinogen concentration are all acute phase proteins, it follows logic

that the statistically significant change in fibrinogen might not have been brought about by the supplements, but an inflammatory response.

Fibrin network lysis rate of day 1, 8, 36 and 43 showed the same pattern and similar lysis rates, while day 22 reached a lower end-point absorbance, which cannot be explained by the fibrinogen concentration. It can be noticed that the network fibrin content of day 22 was lower than that of all the other visits, and this could explain why day 22 end-point absorbance was lower than that of the other visits. Most importantly, there was no significant change from day 8 and day 36.

5.3.1.3 <u>Changes in the lipid profile of the placebo supplement study group</u>

No statistically significant changes were measured within the lipid variables of the placebo group, because this study group consumed placebo which was not supposed to affect the lipid variables.

5.3.2 The Acetate-Propionate (50/50) Supplement Intervention Study Results

This group consumed placebo from day 1 to day 8, followed by the acetate-propionate supplements until day 36, and then consumed placebo for the last week.

5.3.2.1 <u>Changes in the general heath profile of the acetate-propionate (50/50)</u>

<u>supplement study group</u>

No significant changes were observed in any of the general health indicators of the acetate/propionate (half-half) supplemented group over the duration of the study. This can be viewed as positive because no changes were expected in these variables since these variables were only used to assess general health status of the volunteers.

5.3.2.2 <u>Changes in the haemostatic profile of the acetate-propionate (50/50)</u>

<u>supplement study group</u>

A statistically significant decrease in factor VIII activity, antithrombin III and an increase in fibrinogen concentration were measured within this group from baseline to end of supplementation (day 8 and day 36). No statistically significant changes in any of the haemostatic variables were measured. The change in factor VIII could be viewed as positive because some studies have suggested that an increase in factor VIII is associated with increased risk of coronary heart disease development. The changes in ATIII and fibrinogen could render the blood more coagulable and the individual at higher risk of development of cardiovascular complications. This is only supported if the assumption that a decreased ATIII makes the blood more coagulable. An increase in fibrinogen was not accompanied by the high CRP and factor VIII, as was the case with the placebo group, thus indicating that the change is not an

acute phase response. There were no statistically significant changes in the fibrin network architecture, as was seen with Veldman *et al*. (1999) but the discrepancy is caused by the amount of acetate fed and the fact that in this study propionate was also included in the supplements. These changes are not clinically significant.

Network lysis rate of day 8, 22, and 36 showed similar rate of lysis, while day 43 reached the highest end-point absorbance, contrary to day 1 which reached the lowest. The network fibrin content of day 1 was the lowest of all the visits, while day 43 had a couple of samples with high network fibrin content, this is reflected by the high standard deviation. In contrast to day 8's fibrin network content that had a low standard deviation, indicating that the range for this visit was small. Most importantly day 8 and day 36 showed no significant change.

5.3.2.3 <u>Changes in the lipids profile of the acetate-propionate (50/50) supplement study group</u>

Triglyceride levels showed a statistically significant increase from start to the end of supplementation. An increase in TG could be viewed as making the individual susceptible to cardiovascular events, but in this case the increase is still well below the high risk cut-off level of 2 mmol/L. Also, a significant decrease in LDL-C was measured from baseline two (day 8) to the end of the supplementation phase (day 36). In comparison with TC levels (LDL-C/TC X 100), the LDL-C fraction dropped from 64%

(day 8) to 57% (day 36). This in itself may have some clinical significance. The rise in HDL-C was small and statistically non-significant, but with the decrease in LDL-C caused the %HDL-C to rise from 26% to 30%. The statistically significant decrease in LDL-C and a concomitant rise in %HDL-C (HDL-C/TC X 100) can be considered as positive changes, thus reducing the risk of cardiovascular events. These changes can be related to those found by Veldman *et al.* (1999) where acetate and pectin were given as supplements, and Venter *et al.* (1997) where feeding a western diet together with 2% propionate showed an increase in HDL-C.

5.3.3 The High-Acetate Supplement Intervention Study Results

This group consumed placebo from day 1 to day 8, followed by high-acetate supplements till day 36, and then consumed placebo for the last week.

5.3.3.1 <u>Changes in the general health profile of the high-acetate supplement study group</u>

No significant differences in any of the general health indicators of the high acetate supplemented study group were measured, from the start to the end of supplementation, as indicated no changes were expected.

5.3.3.2 <u>Changes in the haemostatic profile of the high-acetate supplement study group</u>

The changes that affect the cardiovascular disease risk profile positively are, a decrease in factor VII, factor VIII, and fibrin monomers, while a decrease in AT III is unknown, compaction's increase affects the risk profile negatively. The more compact network would have a high degree of cross-linking, thus will not allow easy access for fibrinolytic enzymes (Nair & Shats, 1997; Blombäck *et al.*, 1992). The significant results within haemostatic variables of this subject group are difficult to interpret, mainly due to the fact that some changes could be considered as beneficial (the decrease in FM levels), and the others as less beneficial (an increase in compaction). It will be necessary to weigh these changes against each other, without speculating too much. Though changes were not found in the fibrin network structure as in Veldman *et al.* (1999), but this group showed more changes in the haemostatic variables than the acetate-propionate supplement group. It must be remembered that the supplement of this study does not produce the same SCFAs ratio as pectin. No other significant changes were measured within this subject group.

Network lysis rate of all the visits showed a similar rate of lysis with no statistically significant changes amongst them. Day 43 showed a high end-point absorbance because it had the highest fibrin network content and had a few samples with high fibrin network content values as

reflected by the standard deviation and raw data. Day 22's lysis rate seemed to increase periodically and it reached plateau after all the other visits and it had the second highest end-point absorbance which makes sense because this visit had the second highest fibrinogen concentration. The change in compaction was small and therefore was not reflected in the network lysis rate.

5.3.3.3 <u>Changes in the lipids profile of the high-acetate supplement study group</u>

Total cholesterol within this supplemented group showed a statistically significant increase of approximately 4.2%, compared to no difference within the placebo group. This increase was caused by a small statistical non-significant increase in LDL-C (8%) and HDL-C (5.2%), while the %HDL-C remained constant. At first glance the change looks negative but when examined closer the change is not so cut and dry. These results are a bit tricky to evaluate since a positive and a negative change has taken place. When looking at the %HDL-C and %LDL-C (both relative to TC) there is not much change in these variables. A decrease of approximately 4.2% should have a clinical impact, especially if other variables followed this route. The difference, however, is very small when compared to clinical significance. It is interesting to note the statistical non-significant decrease in NEFA levels (of 25%). This change is expected and ascribed to increase short-chain fatty acid circulation. It is this decrease in the NEFA concentration that was believed to lower

circulation plasma fibrinogen concentrations (Veldman *et al.*, 1999). However, this hypothesis could never be proven. No other significant changes of interest in any of the lipid variables within this group were observed.

CHAPTER 6

CONCLUSION AND RECOMENDATIONS

6.1   INTRODUCTION

Coronary heart disease and stroke are considered as degenerative western diseases which in most cases can be prevented by a change in lifestyle, for example diet, physical activity and psychological well being. Africans have the lowest incidence of these westernized disorders, in comparison to other South African population groups. This is thought to be due to the prudent, low-fat high fibre diet which Africans consume. The protection seems to centre on and around the intake of dietary fibre, but the exact mechanism of action is not known. Dietary fibre is fermented in the large gut to short-chain fatty acids (acetate, propionate and butyrate). It is hypothesized that these SCFAs may, in part, contribute in some way to the beneficial effects of dietary fibre intake on human metabolism.

The aim of this study was to test the effects of different combinations of short-chain fatty acids on some metabolic risk markers generally associated with the development of cardiovascular heart disease in westernised African men. In this study a group of westernised African male volunteers were recruited and fed two different combinations of short-chain fatty acids, as well as a placebo supplement, for a period of six

weeks. Blood was drawn at different intervals and analysed to assess changes that were brought about by the different supplements. Focus was mainly on the differences between start of supplementation (day 8) and end of supplementation (day 36).

The conclusion of the baseline characteristics of the entire study group will be presented first, followed by the intervention study, where the conclusion of the different study groups will be presented, ending with the recommendations.

6.2 BASELINE CHARACTERISTICS OF THE ENTIRE STUDY GROUP

The characteristics of this group of subjects reflect a group of people without current metabolic abnormalities, as reflected by the baseline results and discussions. This could be ascribed to the strict inclusion criteria adhered to during the recruitment phase. The anthropometrical measurements reflect that this group of subjects were not clinically obese, while the blood pressure and blood counts were also within range for this population.

The haemostatic measurements were within the reference ranges for this subject group. The lipid profile of this group reflected a normolipidaemic group with a slight change to the westernised lipid profile though not pronounced.

There was no significant changes, statistical or clinical, observed between the two baselines. This indicated the placebo supplement was successful in establishing a stable base line.

6.3   INTERVENTION STUDY

The baseline results of the three study groups were not statistically and clinically different, indicating that any other significant changes cannot be attributed to bias, because of a different baseline.

6.3.1 The Placebo Supplement Intervention Study Results

The supplements did not affect the anthropometrical measurements, blood pressure, blood cell counts and CRP as none of them showed a statistically and clinically significant change from the beginning of the intervention period to the end of the study.

The placebo supplement did not affect the haemostatic variables including fibrin network architecture variables. The statistically significant increase in fibrinogen might have been brought about by an inflammatory reaction because CRP and factor VIII were also high for that period, though not statistically significant. The CRP and Factor VIII changes were also not clinically significant.

The lipid variables of the placebo group were not affected by these supplements. This is viewed as being positive since no significant changes were expected in any of the measured variables of this group of subjects.

6.3.2 The Acetate-Propionate (50/50) Supplement Intervention Study Results

The general health indicators were not affected by the supplements; this was expected since these were only used as a measure of general health.

A statistically significant decrease in factor VIII can be viewed as a positive change, while an increase in fibrinogen is viewed as negative changes.

Though statistically significant these two variables are not clinically significant. The decrease in ATIII has to be evaluated further, so as to determine if a decrease or an increase can be associated with increased risk of CHD.

A statistically significant decrease in LDL-C from 64% to 57%, combined with a statistically non-significant increase in HDL-C from 26% to 30% can be considered positive effects. While a decrease in triglycerides is negative, but it was well below the cut-off point

This supplement tended to have affected more lipid variables than the high acetate group. The acetate–propionate supplement did not have an effect on the fibrin network structure variables, but had an effect on other haemostatic variables together with lipid variables.

6.3.3 The High-Acetate Supplement Intervention Study Results

The general health indicators were not affected by the supplements; this was expected since these were only used as a measure of general health.

A statistically significant decrease in factor VII, VIII and fibrin monomers are considered to lower the risk of cardiovascular events. While a increase in compaction tend to increase the risk of cardiovascular events. The contribution of ATIII to CHD risk has still to be determined.

There was a statistically significant increase in total cholesterol of 4.2%, this was caused by an increase in LDL-C and HDL-C of 8% and 5.6% respectively.

This study group showed more changes in the haemostatic variables than the acetate-propionate supplemented group. The high acetate supplements showed only one significant change in the fibrin network structure studies (which was negative), but had an effect on other haemostatic variables and only a single change in lipid variables.

6.3.4 The overall general conclusion

The placebo supplement did not affect the haemostatic variables including fibrin network architecture variables. The acetate-propionate (50/50) supplement affected more lipid variables than the high acetate group. While the high acetate study group showed more changes in the haemostatic variables than the acetate-propionate supplemented group.

6.4   RECOMMENDATIONS

6.4.1 Future research

Is it not time to look closer at fibrin network structure studies for answers for hypercoagulation patients, where a cause for the increased risk for thrombosis is not determined by the tests that are currently done? The researcher suggests a multi-centre study to investigate the effect of fibrin network structure on the risk for hypercoagulation, thus determining if fibrin network structure studies are an independent risk marker or not. This study will also help to determine the cut-off point for risk and on the other assist with the standardisation of the different methods used for fibrin network structure studies.

Another study would be to determine the effect of SCFA and fibre on people who are at risk and even have had an event so as to assess the effect under these "abnormal" conditions.

6.4.2 Dietary recommendations and possible clinical use

On the basis of the results obtained and results from other similar studies it has become very important to make the general public aware of the importance of fibre in the diet, notwithstanding the importance of a balanced diet. For those individuals who may have allergies or are intolerant to certain types of fibre may look towards SCFA supplements as a substitute, though it does not give all the benefits of fibre.

CHAPTER 7

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APPENDIX: A

CONSENT FORM

THE EFFECTS OF SHORT-CHAIN FATTY ACIDS ON HAEMOSTATIC RISK FACTORS FOR CARDIOVASCULAR HEART DISEASES IN WESTERNISED BLACK MEN

DECLARATION BY OR ON BEHALF OF THE PARTICIPANT

I, ... undersigned, ... of .... … (Address)
Identity number: …………………………………………

A   I confirm that:

1.   I have been asked to participate in the above-mentioned research project, carried out by the Fibrinogen Unit, Technikon Free State and University of the Orange Free State.

2.   The information including the purpose of the study, advantages and disadvantages have been completely explained to me.

3.   I give my permission for the use of the results obtained in this research project for publication purpose, thus making other scientists aware of the findings as long as my anonymity is protected at all times. The information obtained will be confidential.

4.   It was clearly explained to me that I can refuse to participate in this study or I can withdraw my permission to participate at any time. If I refuse or withdraw, I will not be disadvantaged in any way and it will not be held against me.

5.   The   information   was   explained   to   me   by .... (name   of   interviewer)   in .... (language) and I confirm that I have good command of this language and understood the explanations. I was also given the opportunity to ask questions on things I did not understand, and I can also ask further questions at any time during the project.

6.   No pressure was applied on me to take part in this research project.

B   I hereby agree voluntarily to take part in this research project.

Signed/confirmed at .... on 2000

....   (signature)   ....
           Participant   Witness

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DECLARATION BY OR ON BEHALF OF RESEARCHER

I, …………………………………………………………………………..…………., declare that:

1.   I have explained the information in this document and about the project to …………………………….. …………………………..……….. (name of participant).

2   I asked the participant to ask any questions if something was not clear.

3   That the interview was conducted in English, Afrikaans, or ………………….. (language) OR that the interview was conducted in ……………………. (language) with the help of a translator, ………………………………………(name of translator).

Signed/confirmed at ……………………………………….... on ………………………...2000

………………………………………..   (signature)   ………………………………………
Researcher or his representative   Witness

DECLARATION BY TRANSLATOR

I, …………………………………………………………………………..…………., declare that:

1   I have translated the content of this document from English/Afrikaans into ………………….. (language) to ………………………………………..…. (name of participant) and that I have translated the participant's questions to the researcher or his/her representative as well as the answers of the latter to the participant.

2   That the information that I have so translated was a correct interpretation of what I was asked to translate

Signed/confirmed at ……………………………………….... on ………………………...2000

………………………………………..   (signature)   ………………………………………
           Translator   Witness

FOROMO YA TUMELLO

FATTY ACIDS TSA KETANE TSE KHOTSWANE DI TSHWENYA JWANG MATSHWAO A LEFU LA PELO MO BANNENG BA BATHO BANTSHO

BOIKANO KA, KAPA MO SEBAKENG SA MO NKA KAROLO

Nna, …………………………………………………………………………………… mo nka karolo, wa ……………………………………………………………………………………………… ………… ……………………………………………………………………………………………… …. (aterese)
Nomoro ya boitsibiso: …………………………………

A   Ke dumela hore:

1.   Ke kopilwe hore ke nke karolo resetjheng eo ho buiwang ka yona hodimo, e etswa ke Fibrinogen Unit, Technikon ya Foreisetata le University ya Foreisetata.

2.   Ke hlaloseditswe maikemisetso, melemo le dikotsi tsa resetjhe ena ka botlalo.

3.   Ke fana ka tumello ya ka hore diphetho tsa resetjhe ena di sebedisetswe ho ngola atikele etla tsibisa bo rra saense ba bang ka diphitlhelelo tsena, empa dipheto di tla ba sephiri, feela di tla sebediswa fa lebitso la ka le sa hlahe ka nako tsohle.

4.   Ke hlaloseditswe hore nka hanna ho nka karolo, kapa nka hula tumello ya ka ya ho nka karolo ka nako e ngwee le e ngwee, mme sena ha se na ho sebediswa kgahlano le nna.

5.   Ke   hlaloseditswe   ka   resetjhe   ke ………………………………………………………………….. (lebitso la mohlalosi) ka ………………………(puo) ke a dumella hore puo ena ke a e utlwisisa le hona ho e tseba. Ke fuwe sebaka sa ho botsa dipotso ka dintho tseo ke neng ke sa di utlwise, ke utlwisisa hore nka botsa ka nnako e ngwee le e ngwee ha resetjhe e tsweletse.

6.   Ha ke a bewa ka tlasa khatelelo hore ke nke karolo mo resetjheng ena.

B.   Ke dumella ka ho rata ha ka ho nka karolo mo resetjheng ena.

E saennwe …………………………………………… . (sebaka), ka la ……………………….. 2000

…………………………………………..   ………………………………………….
        Mo nka karolo   Paki

BOIKANO KA KAPA MO SEBAKENG SA MORESETJHI

Nna, ………………………………………………………………………………………………, ke ikana hore:

1. Ke hlaloseditse …………………………………………………………………… (lebitso la mo nka karolo) ka resetjhe le foromo ena.

2. Ke bolleletse mo nka karolo ho botsa dipotso ha e ba a sa utlwisise

3. Dipotso le thlahiso lesedi di ne di nne di le ka English, Afrikaans, kapa ………………………(puo); kapa dipotso di ne di botswa ka ……………………..(puo) ka thuso ya mofetoledi, ………………………
……………………….(lebitso la mofetoledi).

E saennwe ……………………………………… . (sebaka), ka la …………………………….. 2000

………………………………………   ………………………………………….
         Moresetjhi   Paki

BOIKANO KA MOFETOLEDI

Nna, ………………………………………………………………………………………………, ke ikana hore:

1. Ke fetoletse ntshetso lesedi le dipotso ho tswa ho English/Afrikaans ho ya ho ………………………... (puo) ho ……………………………………………………..(lebitso la mo nka karolo), le dikarabo ho tswa ho moresetjhi ho ya ho mo nka karolo.

2. Ke fetoletse di ntho tseo ho neng ho thwe ke di fetolele hantlhe

E saennwe ……………………………………… . (sebaka), ka la …………………………….. 2000

………………………………………   ………………………………………….
         Mofetoledi   Paki

APPENDIX: B

TECHNIKON FREE STATE FIBRINOGEN UNIT

SCFA PROJECT: RECRUITMENT QUESTIONNAIRE

DATE: _____/_____/_____

INTERVIEWER: _____

SURNAME   **AND**   **INITIALS**:
_____

HOUSE   **DOCTOR**:
_____

PATIENT:
NAME:_____

ADDRESS:_____

        _____

        _____

SECTION/DEPARTMENT:_____

TEL: _____

AGE: _____years_____months

SMOKING HABITS:

                    YES

                    NO

BODY MASS: _____cm

LENGTH: _____kg

BLOOD PRESSURE: _____/_____

ACTIVITY LEVEL:

       INACTIVE

       MEDIUM ACTIVE

       ACTIVE

FAMILY HISTORY:

       CORONARY HEART DISEASE   _____
       DIABETES MELLITUS   _____
       HYPERCHOLESTEROLAEMIA   _____
       OTHER (specify):

       _____

MEDICAL HISTORY:

ANGINA/CORONARY HEART DIASEASE   _____

       MYOCARDIAL INFARCTION   _____
       STROKE   _____
       BYPASS   _____
       BLOOD CLOTS   _____
       HIGH BLOOD PRESSURE   _____
       DIABETES TYPE I/II   _____
       FAMILIAL CHOLESTEROL   _____

ANY CHRONIC DISEASES MEDICATION:

       YES

       NO

SPECIFY:_____

HOW OFTEN DO YOU USE ALCOHOL:

DAILY

WEEKLY

MONTHLY

OTHER: _____

BLOOD TESTS

BLOOD CHOLESTEROL: _____

FASTING GLUCOSE: _____

PLASMA FIBRINOGEN: _____

FOR OFFICE USE ONLY:

SUBJECT: APPROVED   REJECTED

ASSIGNED SUBJECT NO:

APPENDIX:C

QUESTIONNAIRE FOR SUBJECTS WHO COMPLETED THE STUDY

Please answer the following questions regarding your participation in the research study:

Respondent number: _____

Employee Number: _____

1. Did you experience any vomiting during the study?

        1. Yes   2. No

If yes, how many times?

        1. After each meal   2. Once a day   3. Once a week

          4. Other

2. Did you experience any flatulence during the study?

        1. Yes   2. No

If yes, how many times?

        1. After each meal   2. Once a day   3. Once a week

          4. Other

3. Did you experience any stomach cramps during the study?

        1. Yes   2. No

If yes, how many times?

        1. After each meal   2. Once a day   3. Once a week

          4. Other

4. Did you experience any changes in your lifestyle during the study?

        1. Yes   2. No

If yes, describe the changes:

5. Did you experience any constipation during the study?

        1. Yes   2. No

If yes, how frequent?

        1. After each meal   2. Once a day   3. Once a week

        4. Other

6. Did you experience an increased appetite during the study?

        1. Yes   2. No

If yes, explain:

7. Did you experience any changes in your alcohol consumption during the study?

        1. Yes   2. No

If yes, to what extent?

8. Did you use any medication/supplements during the study?

        1. Yes   2. No

If yes, what is the name of the medication/supplement you used?

If yes, how many times did you use this medication/supplement?

        1. After each meal   2. Once a day   3. Once a week

           4. Other

and for how long (days)?

9. Did you consume all of the experimental capsules every day?

        1. Yes   2. No

10. Was the amount of capsules consumed acceptable?

        1. Yes   2. No

11. Would you be willing to consume these capsules daily if they are considered as healthy?

        1. Yes   2. No

12. Did you experience any other side-effects of the supplement during the study?

        1. Yes   2. No

If yes, please specify?

If yes, how frequent?

        1. After each meal   2. Once a day   3. Once a week

        4. Other

13. Did you experience any positive effects on your health during the study?

               1. Yes   2. No

If yes, explain.

14. Do you have any other comments you would like to make regarding the study?

The research team would like to thank you for your co-operation during the study. The project is very important for gaining new scientific knowledge.

APPENDIX D