THE EFFECT OF NUTRITION ON RISK FACTORS FOR CORONARY HEART DISEASE

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AFRIKAANSE TITEL

Die effek van voeding op risikofaktore vir kardiovaskulêre siekte

OPSOMMING

Motivering: Kardiovaskulêre siekte (KVS), insluitende koronêre/iskemiese hartsiekte, angina pektoris en beroerte, is wêreldwyd en ook in Suid-Afrika, een van die belangrikste oorsake van mortaliteit en morbiditeit. Daar word voorspel dat in 2020, KVS die belangrikste oorsaak van sterftes in ontwikkelde sowel as ontwikkelende lande sal wees. KVS kan deur verskeie faktore veroorsaak word. Hierdie faktore word in lewenstyl-, gedrags- of ander nie-veranderbare risikofaktore, wat weer verskeie fisiologiese of metaboliese risikofaktore tot gevolg kan hê, verdeel. Dieetintervensie, tesame met ander lewenstylveranderinge, speel 'n noodsaaklike rol in die behandeling van sommige van hierdie risikofaktore. Daar bestaan egter nog onsekerhede, teenstrydighede en 'n tekort aan data in die litaratuur aangaande sommige van hierdie dieetfaktore se effekte op verskeie risikofaktore vir KVS.

Doelwitte: Die hoofdoel van hierdie studie was om die effekte van 'n paar voedingspraktyke waaroor daar tans in die literatuur en dieetkundepraktyk kontroversie bestaan, op die relevante risikofaktore vir KVS te ondersoek. Die voedingspraktyke was alkoholinname, suiker (sukrose)–inname, lesitien en dieetvesel. Die dieetvesel was afkomstig van twee bronne, naamlik koringsemels en droëbone en is in twee nuwe produkte of "funksionele voedsels" (hoëvesel gebakte produk en geëkstrueerde droëboneproduk) getoets. Om hierdie hoofdoel te verwesenlik, is die volgende doelwitte vir elk van die vyf afsonderlike studies wat in hierdie proefskrif gerapporteer word, gestel:

- Om die gemiddelde alkoholinname en verband daarvan met serumlipoproteïene en bloeddruk in blanke Suid-Afrikaners te beskryf. Hierdie proefpersone het aan die VIGHOR-studie, 'n ewekansige dwarsdeursnit epidemiologiese studie, deelgeneem;

- om die verband tussen toegevoegde suikerinname en liggaamsmassa-indeks (LMI), asook die effekte van toegevoegde suikerinname op makro- en mikronutrientinnamepatrone van

dieselfde blanke Suid-Afrikaners wat aan die VIGHOR-studie deelgeneem het, te ondersoek;

- om die effekte van lesitien op serumlipoproteïen-, plasmafibrinogeen- en makromolekulêre proteïenkompleks (MPK)-vlakke met behulp van 'n dubbel-blinde studie wat vir moontlike indirekte effekte gekontroleer het, te ondersoek;

- om die effekte van hoëvesel- (koringsemels) *vs* laevesel-maaltye op postprandiale serumlipoproteïen- en plasmafaktor VII koagulantaktiwiteitresponse met behulp van 'n ewekansige oorkruisstudie te ondersoek; en

- om die effekte van die insluiting van geëkstrueerde droëbone in die dieet op serumlipoproteïene, plasmafibrinogeen, plasmaviskositeit en plasminogeenaktiveerderinhibeerder 1 (PAI-1)-vlakke met behulp van 'n ewekansige gekontroleerde oorkruisstudie te ondersoek.

Metodologie: Hierdie proefskrif bestaan uit drie gepubliseerde artikels en twee manuskripte wat vir publikasie voorgelê is. Die leser word verwys na die opsommings aan die begin van elke publikasie in hoofstukke 3-7 vir 'n beskrywing van die proefpersone, studie-ontwerp en analitiese metodes wat in elke studie gebruik is. Om die verwantskap tussen alkohol- en suikerinname met KVS-risikofaktore te ondersoek, is data wat van 'n dwarsdeursnit epidemiologiese studie, die VIGHOR-studie, verkry is, gebruik. Die verwantskappe is met behulp van parsiële korrelasies bepaal sodat daar vir faktore wat moontlik die resultate kon beïnvloed, gekontroleer kon word. Om die invloed van lesitien, koringsemels en geëkstrueerde droëbone te evalueer, is voedingsingrepe onder gekontroleerde toestande by homogene groepe mans uitgevoer. Effekte op beide die lipoproteïen- en hemostatiese risikofaktore is met gestandaardiseerde metodes gemeet.

Resultate en gevolgtrekkings van die individuele studies:

- Analises van die VIGHOR-data het getoon dat alkoholinname 'n positiewe effek op hoëdigtheidslipoproteïencholesterol (HDL-C)-vlakke in beide mans en vroue gehad het. 'n Klein negatiewe effek op bloeddruk is egter in mans waargeneem. Die inname van alkohol om teen KVS te beskerm, word dus nie aanbeveel nie, omdat dit met beide risiko en beskermende effekte teen KVS geassosieer kan word. Alkohol maak egter deel van die

Suid-Afrikaanse lewenstyl uit en vir diegene wat wel alkohol gebruik, behalwe individue met alkoholverwante siektetoestande, kan ligte tot matige inname tesame met die beheer van ander KVS-risikofaktore aanbeveel word.

- Geen verband tussen daagliks toegevoegde suikerinname en LMI kon in die VIGHOR-proefpersone aangetoon word nie. Suikerinname is negatief met vetinname geassosieer. In mans was suikerinname nie 'n groot risiko vir gebrekkige mikronutriëntinname nie. Die vroue, moontlik as gevolg van hul lae energie-inname, was meer vatbaar vir gebrekkige nutriëntinname met verhoogde suikerinname, veral wat betref dieetvesel, yster, magnesium, koper, tiamien en vitamien B_6. Die vermyding of beperking van suikerinname sal moontlik nie die reeds nutriënt-onvoldoende diëte herstel nie en mag selfs tot verhoogde vetinname lei. Die boodskap aangaande suikerinname vir hierdie populasie en ander wat "westerse' diëte begin volg, behoort 'n positiewe een te wees, naamlik dat toegevoegde suiker in matige hoeveelhede ingeneem kan word. Vroue wat lae-energie diëte volg, moet egter versigtig wees om nie nutriënt- en veselryke voedsel met suikerbevattende voedsels te vervang nie.

- In die studie waarin sojaboon-lesitien se effekte getoets is, het plasmalinoleïensuur betekenisvol tydens lesitienbehandeling verhoog, wat op inskiklikheid tot die dieetbehandeling dui. Lesitienbehandeling het egter geen onafhanklike effekte op serumlipoproteïen-, plasmafibrinogeen- of MPK-vlakke in hierdie hiperlipidemiese manlike proefpersone gehad nie. Die resultate dui daarop dat die daaglikse inname van 20g lesitien nie as 'n hipolipidemiese middel aangewend kan word nie.

- Hoëvesel-maaltye het meer geleidelike serumtrigliseried- (TG) en retinolpalmitaat- (merker vir chilomikrone en hul oorblyfsels) responskurwes in vergelyking met laevesel-maaltye tydens 'n postprandiale studie in normolipidemiese mans veroorsaak. Dit het tot betekenisvolle hoër TG- en retinolpalmitaat- en laer HDL-C-vlakke na drie ure gelei. Voordat 'n finale gevolgtrekking oor hierdie moontlik nadelige effek van vesel gemaak kan word, behoort die resultate ook deur ander postprandiale studies bevestig te word. Die moontlik meganismes wat vir dié bevindinge verantwoordelik is, behoort verder ondersoek te word. 'n Positiewe bevinding wat wel uit hierdie studie na vore gekom het, was dat die inname van normale fisiologiese hoeveelhede vet in 'n maaltyd, soos in hierdie studie

gebruik (30.5g vet/maaltyd), nie plasmafaktor VII geaktiveer het nie, selfs al het TG-vlakke betekenisvol verhoog.

- Inskiklikheid tot die inname van geëkstrueerde droëbone was relatief goed, aangesien die gemiddelde inname 91.9g/dag (83.5%) was. Geëkstrueerde droëbone het geen effek op serumlipoproteïen-, plasmafibrinogeen- en plasmaviskositeitsvlakke gehad nie, maar het 'n positiewe effek op PAI-1 gehad. PAI-1-vlakke was betekenisvol laer na die inname van geëkstrueerde droëbone in vergelyking met die kontroleperiode, wat voordelige effekte vir fibrinolitiese aktiwiteit mag inhou.

Gesamentlike bespreking: 'n Kritiese evaluering van die aanbevelings wat gebaseer is op resultate wat in die vyf afsonderlike studies verkry is, het getoon dat almal versoenbaar is met die nuutste konsensus dieetriglyne vir die behandeling van die algemene hiperlipidemieë in Suid-Afrika. Die studie het egter aangetoon dat meer navorsing oor die postprandiale effekte van dieetvesel op die lipiedmetabolisme gedoen moet word. Die studie het verder meer lig gewerp op die potensiële voordelige effekte van dieetbehandeling op die hemostatiese sisteem. Dit beklemtoon die belangrikheid van meer navorsing in hierdie veld voordat sinvolle dieetriglyne vir die behandeling van hiperkoaguleerbaarheid saamgestel kan word. 'n Belangrike bydrae van hierdie studie is dat dit aantoon hoe nuwe "funksionele voedsels" (die hoëvesel gebakte produk en die geëkstrueerde droëboneproduk) se effekte op metaboliese risikofaktore van KVS in wetenskaplik-verantwoordbare goed beplande gekontroleerde studies geëvalueer moet word voordat gesondheidsaansprake oor hierdie voedselprodukte gemaak kan word.

Sleutelwoorde: alkohol, dieetsuiker, nutriëntinname, lesitien, fosfolipiede, dieetvesel, droëbone, lipoprotein, bloeddruk, liggaamsmassa-indeks, fibrinogeen, makromolekulêreproteïenkompleks, postprandiale lipidemie, postprandiale faktor VII, viskositeit, plasminogeenaktiveerderinhibeerder 1.

SUMMARY

Motivation: Cardiovascular disease (CVD), including coronary/ischaemic heart disease, angina pectoris and stroke, is one of the leading causes of mortality and morbidity world-wide as well as in South Africa. It is predicted that in 2020 CVD will become the most important cause of death and morbidity in developed and developing countries. Numerous factors can contribute to the development of CVD. These factors can be divided into lifestyle or behavioural and other non-modifiable risk factors that may result in various physiological and metabolic risk factors. Dietary intervention, together with other lifestyle changes, form an important part of the management of some of these risk factors. There are, however, still some uncertainties, inconsistencies and a lack of data in the literature regarding the effects of some dietary factors on various CVD risk factors.

Objectives: The main objective of this study was to examine the effects of a few dietary factors on the relevant CVD risk factors about which controversies still exist in the literature. The dietary factors were alcohol intake, sugar (sucrose) intake, lecithin and dietary fibre. The dietary fibre was obtained from two sources, namely wheat bran and dry beans that were tested in two new products or "functional foods" (high fibre baked product and extruded dry bean product). To attain this main objective, the following objectives for each of the five separate studies were stated:

- To describe the mean alcohol consumption and its relationships with serum lipoproteins and blood pressure in white South Africans. These subjects participated in the VIGHOR study, a randomised cross-sectional epidemiological study;

- to investigate the relationship between added sugar consumption and body mass index (BMI) and the effects of added sugar consumption on the macro and micronutrient intake patterns in the same white South Africans who participated in the VIGHOR study;

- to examine the effects of lecithin on serum lipoprotein, plasma fibrinogen and macro molecular protein complex (MPC) levels in a double-blind study which controlled for possible indirect effects;

- to determine what the effects of high fibre (wheat bran) *vs* low fibre meals would be on postprandial serum lipoprotein and plasma factor VII coagulant activity responses in a randomised cross-over study; and

- to examine the effects of the inclusion of extruded dry beans in the diet on serum lipoprotein, plasma fibrinogen, plasma viscosity and plasminogen activator inhibitor (PAI-1) levels in a randomised controlled cross-over study.

Methodology: This thesis consists of three published papers and two manuscripts submitted for publication. The reader is referred to the abstracts at the beginning of each separate paper in chapters 3-7, for a description of the subjects, study design and analytical methods used in each study. To examine the relationships between alcohol and sugar intake with CVD risk factors, data from a cross-sectional epidemiological study, the VIGHOR study, were used. These relationships were estimated by using partial correlations to control for possible confounding factors. To examine the effects of lecithin, wheat bran and extruded dry beans, nutrition interventions were performed under controlled circumstances on homogenous groups of men. Effects on both the lipoprotein and haemostatic risk factors were analysed by means of standard methods.

Results and conclusions of the individual studies:

- Analyses of the VIGHOR data showed that alcohol consumption had a positive effect on high density lipoprotein cholesterol (HDL-C) levels in both men and women. A small negative effect on blood pressure was, however, observed in men. The consumption of alcohol for cardioprotective purposes is therefore not encouraged, since it can be associated with both risk and protective effects against CVD. Because alcohol forms part of many South Africans lifestyle, for those who do consume alcohol, except for individuals with alcohol-related disorders, light to moderate consumption together with management of other CVD risk factors can be recommended.

- No relationship between daily added sugar intake and BMI was observed in the VIGHOR subjects. Sugar intake was negatively associated with fat intake. In the men, sugar intake did not constitute a major risk for nutrient inadequacy. The women, probably because of their low energy intakes, were, however, more prone to nutrient inadequacies with increased sugar intake, especially with regard to dietary fibre, iron, magnesium, copper,

thiamine and vitamin B_6. Avoidance or restriction of sugar consumption will probably not correct an already nutrient-inadequate diet and may even lead to an increased intake of fat. It is suggested that the message regarding sugar consumption to this population and others moving towards "westernised" diets, should be that added sugars can be consumed in moderation. Women with low energy intakes should be cautious not to displace nutrient- and fibre-rich foods with foods containing large amounts of sugar.

- In the study which examined the effects of soya bean lecithin, lecithin treatment caused significant increased plasma linoleic acid levels, indicating that compliance to the treatment was obtained. Lecithin treatment, however, had no independent effects on serum lipoprotein, plasma fibrinogen of MPC levels in these hyperlipidaemic men. The results therefore suggest that the daily intake of 20g lecithin cannot be used as a hypolipidaemic agent.

- High fibre meals caused more gradual serum triglyceride (TG) and retinyl palmitate (marker for chylomicrons and their remnants) response curves compared to low fibre meals in a postprandial study in normolipidaemic men. This resulted in significantly higher levels of TG and retinyl palmitate and lower HDL-C levels after three hours. Before final conclusions regarding this possible detrimental effect of fibre can be made, these findings need to be confirmed by other postprandial studies. The possible mechanisms responsible for these findings need to be further examined. A positive finding from this study was that the intakes of normal physiological amounts of fat in a meal, as used in this study (30.5g fat/meal) did not activate plasma factor VII, even though serum TG increased significantly.

- The mean intake of extruded dry beans per day was 91.9g (83.5%), indicating relatively good compliance with the experimental intervention. Extruded dry beans had no effects on serum lipoproteins, plasma fibrinogen and viscosity levels, but indicated a positive effect on PAI-1. PAI-1 levels were significantly lower after the intake of extruded dry beans compared to the control period that might have beneficial effects on the fibrinolytic system.

Combined discussion: A critical evaluation of the recommendations based on the results from the five separate studies indicated that they were all compatible with the latest consensus dietary guidelines for the management of the common hyperlipidaemias in South Africa. However, the study indicated that more research regarding the postprandial effects of dietary

fibre on the lipid metabolism is needed. The study further contributed to the potential benefits of dietary treatment on haemostatic factors. It also stresses the importance of more research in this field before meaningful dietary guidelines can be made for the management of hypercoagulability. An important contribution of this study lies in the development of capacity to evaluate the effects of new "functional foods" (the high fibre baked product and the extruded dry bean product) on metabolic risk factors for CVD in a scientifically responsible way in well-designed controlled studies before health claims regarding these food products can be made.

Key words: alcohol, dietary sugar, nutrient intake, lecithin, phospholipids, dietary fibre, dry beans, lipoprotein, blood pressure, body mass index, , fibrinogen, macro molecular protein complex, postprandial lipaemia, postprandial factor VII, viscosity, plasminogen activator inhibitor 1

CONTENTS

ABBREVIATIONS

%E	Percentage of the total energy intake
a	Activated
ACAT	Acyl CoA:cholesterol acyltransferase
ADP	Adenosine 5`-diphosphate
APC	Activated protein C
Apo	Apoprotein
AT	Antithrombin
BMI	Body mass index
Ca	Calcium
Cat.no.	Catalogue number
CE	Cholesterol ester
CETP	Cholesterol ester transport protein
CHD	Coronary heart disease
CI	Confidence interval
CV	Coefficient of variance
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
EDRF-NO	Endothelium-derived relaxing factor of nitric oxide
ER	Endoplasmic reticulum
F	Factor
FH	Familial hypercholesterolaemia
FC	Free cholesterol
FDP	Fibrin degradation products
FFA	Free fatty acids
$FVII_c$	Factor VII coagulant activity
GGT	Gamma-glutamyltransferase
GIP	Gastric inhibitory peptide
GPIIb:IIIa	Glycoprotein IIb-IIIa
HDL	High density lipoprotein

HDL-C	High density lipoprotein cholesterol
HL	Hepatic lipase
HMG CoA	Hydroxymethyl-glutaryl-CoA
IHD	Ischaemic heart disease
IDL	Intermediate density lipoprotein
IL	Instrumentation Laboratories
KHS	Koronêre hartsiekte
LCAT	Lecithin : cholesterol acyltransferase
LDL	Low density lipoprotein
LDL-C	Low density lipoprotein cholesterol
LDL_{ox}	Oxidized low density lipoprotein
Lp(a)	Lipoprotein (a)
LPL	Lipoprotein lipase
Lyso-PL	Lysophospholipids
MG	Monoglycerides
MPC	Macro molecular protein complex
MRC	Medical Research Council
MUFA	Mono-unsaturated fatty acid
NSP	Non-starch polysacharides
PAI-1	Plasminogen activator inhibitor 1
PDGF	Platelet derived growth factor
PF3	Platelet factor 3
PF4	Platelet factor 4
PGI	Prostacyclin
PL	Phospholipid
Prot	Protein
PS	Protein S
PUFA	Polyunsaturated fatty acids
RDA	Recommended dietary allowance
RNI	Reference nutrient intake
SBP	Systolic blood pressure
SD	Standard deviation
SFA	Saturated fatty acid
SMC	Smooth muscle cells

TC	Total cholesterol
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TG	Triglyceride
tPA	Tissue plasminogen activator inhibitor
TXA	Thromboxane
uPA	Urokinase plasminogen activator
VIGHOR	Vanderbijlpark Information on Gesondheid/Health, Obesity and Risk factors
VIII:vWF	Von Willebrand factor
VLDL	Very low density lipoprotein
VLDL-C	Very low density lipoprotein cholesterol

CHAPTER 1

INTRODUCTION

1. BACKGROUND AND MOTIVATION

In this chapter cardiovascular disease (CVD) as one of the most important causes of mortality and morbidity world-wide as well as in South Africa will be discussed. The causes or risk factors for CVD will be highlighted and the possible role of diet on these risk factors, especially lipoproteins and some haemostatic factors, will be summarised. Five different studies are reported in this thesis in the form of publications. A short motivation for each study will be given. The aims and objectives of the studies will be stated and the structure of the thesis explained.

1.1 The incidence of cardiovascular disease world-wide and in South Africa

CVD, including coronary/ischaemic heart disease (CHD)/(IHD), myocardial infarction, angina pectoris and stroke (definitions for these terms are given in Chapter 2), is one of the leading causes of mortality and morbidity in South Africa (Bradshaw *et al.*, 1995) and world-wide (Murray & Lopez, 1996). In 1990 stroke was the third and IHD the fifth most frequently reported causes of death in South Africa (Bradshaw *et al.*, 1995). In 1990, IHD was world-wide the biggest and stroke the second biggest cause of death (Murray & Lopez, 1996). It is predicted that in 2020, IHD and stroke will remain the two most important causes of death in developed as well as in developing countries (Murray & Lopez, 1996). In South Africa, mortality rates due to IHD are the highest amongst Asians and whites (Figure 1) (Bradshaw *et al.*, 1995). Although CVD is a multifactorial disease, the typical Western diet, high in fat and low in fibre with inadequate micronutrient intakes, followed by many South Africans (Vorster *et al.*, 1997b) could explain these high rates to a certain extent. The high incidence of CVD could have important economic implications for this country. In 1991 it was estimated that CVD, excluding rehabilitation and follow-up visits, costs South Africa between 4.1 and 5 billion Rand (Pestana *et al.*, 1996). It is therefore clear that CVD is a major public health problem in this country.

It is therefore of the utmost importance that this problem be addressed and that strategies to prevent the occurrence of CVD be developed. One such strategy is the assessment and management of the causes or risk factors for CVD.

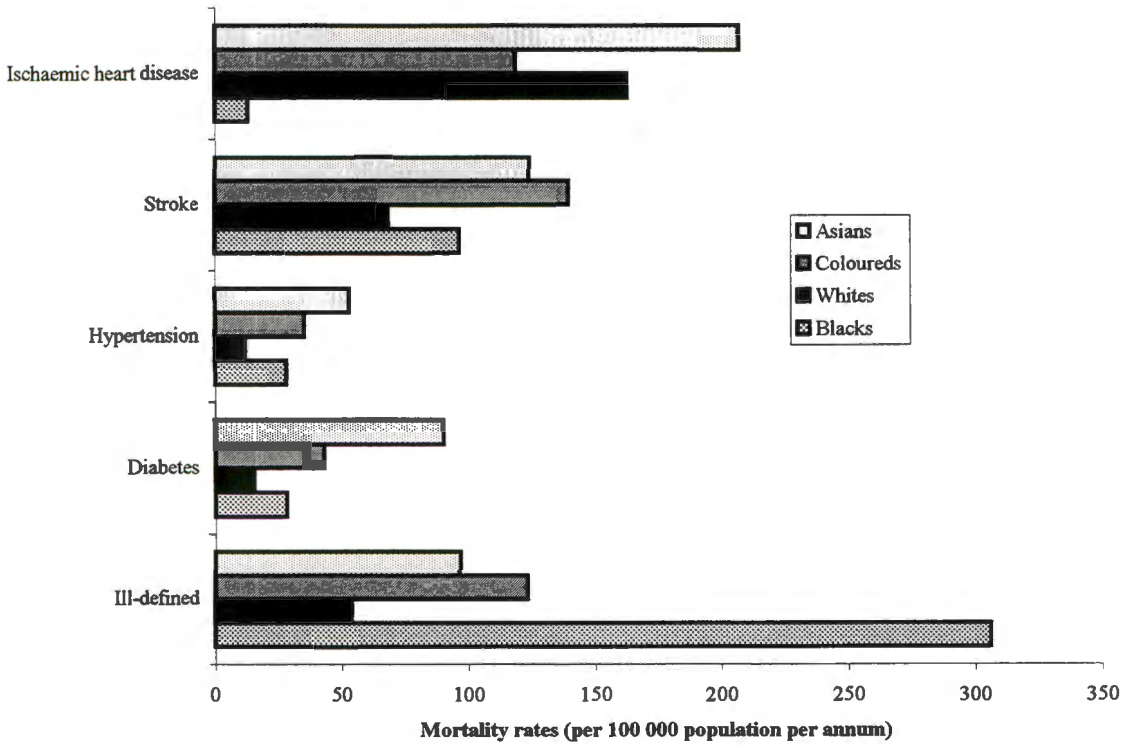

Figure 1 Mortality rates (1984-1986) for ischaemic heart disease, stroke, hypertension, diabetes and ill-defined causes of death (adapted from Bradshaw *et al.*, 1995)

1.2 Risk factors for cardiovascular disease

Prospective epidemiological studies were mainly responsible for the identification of the many different CVD risk factors. Once the risk factors have been identified, intervention studies have to be undertaken to establish a cause-and-effect relationship (Hornstra *et al.*, 1998). Increased low density lipoprotein cholesterol (LDL-C) is probably the most extensively examined risk factor for which a cause-and-effect relationship has been reported (Scandinavian Simvastatin Survival Study Group, 1994; Shepherd *et al.*, 1995). For most of the risk factors cause-and-effect relationships have not been determined. One can, however, not ignore these risk factors, since strong evidence of their relationship with CVD exist. CVD is a multifactorial disease and the risk increases markedly with the addition of each risk factor. It is therefore important that primary prevention and treatment of CVD involve the assessment and management of these risk factors (Marais, 1999). The risk factors can be divided into lifestyle or behavioural and some other non-modifiable risk factors. They are the primary initiating causes of physiological and various metabolic risk factors that can lead to the development of atherosclerosis and ultimately CVD. This causal sequence of events leading to CVD is summarised in Table 1.

Table 1 Risk factors for cardiovascular disease in sequence of events that may lead to the manifestation of cardiovascular disease (Aznar *et al.*, 1988; Danesh *et al.*, 1998; Garrison, 1998; Harris, 1997; Meade *et al.*, 1986; Meade *et al.*, 1996; Pyörälä *et al.*, 1994; Scarabin *et al.*, 1998; Stampfer *et al.*, 1992; Wood *et al.*, 1998; Zilversmit, 1979)

PRIMARY INITIATION RISK FACTORS	PHYSIOLOGICAL AND METABOLIC RISK FACTORS	CVD EVENTS
Lifestyle/Behavioural risk factors: * Smoking * Inactivity * Social and psychological factors * Excess alcohol consumption * Diet high in saturated fat, cholesterol and energy ***Nonmodifiable risk factors:*** * ↑ Age * Gender (men ↑ risk) * Premature menopause without hormone replacement therapy * Family history (myocardial infarction/sudden death before age 55 in male and 65 in female first degree relatives) * Personal history of CHD	*Lipid and lipoprotein risk factors:* * ↑ Total cholesterol* * ↑ Low density lipoprotein cholesterol* * ↑ Triglycerides* * ↓ High density lipoprotein cholesterol* * ↑ Apoprotein B* * ↓ Apoprotein A* * ↑ Lipoprotein (a)* * ↑ Chylomicron remnants* *Haemostatic risk factors:* * Fibrinogen* * Factor VII coagulant activity* * Fibrinolytic activity* * Plasminogen activator inhibitor 1* *Other:* * Overweight/obesity* * Hypertension* * Diabetes mellitus* * Hyperinsulinaemia* * Homocysteine*	* CHD/IHD * Myocardial infarction * Angina pectoris * Stroke

* Can be modified by diet; ↓: Decrease; ↑: Increase; CVD: Cardiovascular disease; CHD: Coronary heart disease; IHD: Ischaemic heart disease

1.3 The possible role of diet on risk factors for cardiovascular disease

Dietary intervention, together with other lifestyle changes, form an essential part of the management of some CVD risk factors, for example hyperlipidaemia, hypertension, diabetes mellitus, obesity (Gotto *et al.*, 1995; Shrapnel *et al.*, 1992; Bradshaw *et al.*, 1995), the fibrinolytic (Mehrabian *et al.*, 1990; Rånby & Sundell, 1992) and coagulation systems (Marckmann *et al.*, 1993; Venter *et al.*, 1992; Vorster & Venter, 1994a). Since the five studies in this thesis mainly examined the effects of dietary factors on lipids and lipoproteins and on haemostatic factors such as fibrinogen, macro molecular protein complex (MPC), plasma viscosity, plasminogen activator inhibitor 1 (PAI-1) and postprandial factor VII coagulant activity ($FVII_c$), the effects of diet on these factors will be briefly summarised in Tables 2 and 3.

The only intervention study that examined the effects of diet on MPC levels is the study of Veldman (1996), where pectin, a soluble dietary fibre component, decreased MPC levels significantly. Lipinski *et al.* (1995) described MPC as a complex that is formed with the fibrin network in blood clots, increasing clot mass and decreasing clot fibrinolysis. It is hypothesised that high MPC levels increase thrombotic risk (Lipinski *et al.*, 1995).

The dietary effects summarised in Tables 2 and 3 are a simplification of the results from a large number of dietary intervention studies. Responses may differ, depending on whether normal or hyperlipidaemic subjects participated, the amounts of nutrients or foods ingested, the type of food being replaced in the diet, or the type of food with which comparisons are made.

Data regarding the effects of diet on haemostatic factors are very limited and inconsistent (Table 2). In most cases only one study on the particular dietary factor has been done. Often, in cases where more than one study was done, the results were conflicting. Even though the effects of diet on lipids and lipoproteins, especially in the fasting state, have been examined extensively, uncertainties regarding some dietary factors still exist (Table 3). There is therefore a need for more studies to examine the effects of diet on these factors.

In order to promote healthy diets as an essential part of the prevention and management programme for CVD in South Africa, it is important that the dietary recommendations are supported by valid and consistent data, targeted at the South African population's specific health issues. In this thesis five studies are reported in which the effects of some dietary

factors were examined where a lack of data, inconsistent results and uncertainties in the literature still existed. In all these studies, white South Africans who are at increased risk for CVD (Bradshaw *et al.*, 1995) participated as experimental subjects.

Table 2 The effects of diet on haemostatic factors

Dietary factor	Haemostatic factors				
	Fibrinogen*	Blood/ plasma viscosity*	Plasminogen activator inhibitor 1*	Factor VII_c	
				Fasting*	Postprandial*#
Energy resulting in weight loss	↓, ↑, ↔	↓	↓	↓	-
Moderate alcohol consumption	↓	-	↑	-	-
High total fat consumption	↑	-	↑	↑	↑
SFA *vs* MUFA *vs* PUFA	-	-	-	-	↔
MUFA	↔	-	↓, ↑	-	-
Fish and fish oil	↓, ↑, ↔	-	↑	-	-
Total dietary fibre	↓, ↔	-	-	↓	↔
Soluble dietary fibre	↓	-	↓, ↔	-	-
Tea	↔	-	↔	-	-
Vegetables and fruit		-	↓	-	-

SFA: Saturated fatty acid; MUFA: Mono-unsaturated fatty acid; PUFA: Polyunsaturated fatty acid; ↑: Increase; ↓: Decrease; ↔: No change

* Summarised from a recent review of the literature by Vorster *et al.* (1997a).

\# Summarised from Mennen *et al.* (1996) and Marckmann *et al.* (1993).

Table 3 The effects of diet on lipids and lipoproteins

Dietary factor	Lipids and lipoproteins				
	TC & LDL-C*	HDL-C*	TG*	Lp(a)♣	Postprandial TG♦
↑ Energy/ overweight	↑	↓	↑	↑, ↔	↑
↑ Total fat: • Moderate fat diet (25-30%E) • Very low fat diet (10%E)	↑ ↓ ↓	↑ ↔ ↓	↑ ↔ ↑	-	↑ - -
SFA	↑	↑	-	↑	↑
Trans fatty acids	↑	↓, ↔	↑	↑	-
MUFA	↔	↔	↔	-	↓
PUFA	↓	↓ (>10-13%) ↔ (<10-13%E)	↓, ↔	-	↓
N-3 fatty acids	↓, ↑, ↔	↓, ↑, ↔	↓	↓, ↔	↓
Cholesterol	↑, ↔	↑, ↔	↔	↔	↑, ↔
Low fat, high carbohydrate diet	↓	↓	↑, ↔	↔	-
Sucrose	↑, ↔	↔	↑, ↔	-	↑
Insoluble fibre	↔	-	-	-	↓
Soluble fibre	↓	↔	↔	-	↓, ↑, ↔
Moderate alcohol consumption	↔	↑	↑	-	-
Protein: • Plant protein	↓, ↔	-	-	-	↔
Coffee: • Boiled • Instant/filtered	↑ ↔	-	-	-	-
Lecithin#	↓, ↔	↑, ↔	↓, ↔	-	-

TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; TG: Triglycerides; Lp(a): Lipoprotein (a); SFA: Saturated fatty acid; MUFA: Mono-unsaturated fatty acid; PUFA: Polyunsaturated fatty acid; %E: Percentage from total energy intake; ↑: Increase; ↓: Decrease; ↔: No change.

* Summarised from a recent and complete review of the literature by Wolmarans (1997).

\# Summarised from Oosthuizen *et al.* (1998).

♣ Summarised from Berglund (1995) and Zock and Mensink (1996).

♦ Summarised from Roche and Gibney (1995) and Bergeron and Havel (1997).

1.4 Motivation for selection of dietary factors

This thesis consists of published papers or manuscripts submitted for publication. Since the relevant literature background for each study is discussed in the papers, only a brief motivation for each chosen dietary factor and the relevant study will be provided here.

1.4.1 Alcohol consumption and its relationship with risk factors for CVD

A U or J-shaped relation between alcohol intake and all-cause mortality exits. Consumption of an average of one to two units of alcohol per day is associated with significantly lower all-cause mortality than the consumption of no alcohol, or the consumption of substantial amounts (Doll *et al.*, 1994; Fuchs *et al.*, 1995). It seems as if light to moderate drinking is associated with the lowest risk, especially from CVD, non-haemorrhagic and ischaemic stroke. Heavier drinking is associated with an increased risk of death from other causes, namely certain cancers, especially breast cancer, cirrhosis and haemorrhagic stroke (Fuchs *et al.*, 1995; Iso *et al.*, 1995; McMichael *et al.*, 1979; Stampfer *et al.*, 1988; Willett *et al.*, 1987). The protective effect of moderate alcohol consumption against CVD has been documented in numerous studies (Camargo *et al.*, 1997a; Camargo *et al.*, 1997b; Iso *et al.*, 1995; Stampfer *et al.*, 1988). The well-known French paradox is an example of a population that, despite their high intake of saturated fat, has a low CVD mortality rate, possibly as a result of alcohol intake (Criqui & Ringel 1994). The beneficial effect of alcohol consumption on CVD could possibly be ascribed to increasing effects on high density lipoprotein cholesterol (HDL-C) (Choudhury *et al.*, 1994; Miller *et al.*, 1988), decreased platelet aggregation and plasma fibrinogen and increased fibrinolytic activity (Vorster *et al.*, 1997a). Current available data does not establish whether there is an upper limit to the amount of alcohol that is protective against CHD (Rehm *et al.*, 1997). The protective effects of alcohol on CVD risk may, however, be counterbalanced by increases in other CVD risk factors, for example blood pressure (Langer *et al.*, 1992) and triglycerides (TG) (Choudhury *et al.*, 1994; Miller *et al.*, 1988) as well as other diseases.

Alcohol consumption in South Africa has been rising. In a South African health review by the Health Systems Trust in 1997, this country ranked 23rd internationally with regard to alcohol consumption, with an annual per capita consumption of 8.5L per adult (Bradshaw, 1997).

Very little is known about alcohol consumption patterns of the different population groups in South Africa, especially with regard to those groups at risk for CVD. The balance between risks and benefits for CVD with alcohol consumption is likely to differ in different populations. White South Africans is one group particularly at risk for CVD (Bradshaw *et al.*, 1995). The white men and women under investigation in this study also followed a typical Western diet, high in fat and low in fibre, with inadequate micronutrient intakes (Vorster *et al.*, 1995), which may increase their risk for CVD. It was therefore decided to describe the mean alcohol consumption and its association with risk factors for CVD in white men and women who participated in the Vanderbijlpark Information on Gesondheid/Health, Obesity and Risk factors (VIGHOR) project.

Results from this study may be used by policy makers on governmental and private levels and by health professionals in the design of appropriate intervention programmes aimed at primary prevention and management of CVD.

1.4.2 Dietary composition and body mass index at different levels of added sugar consumption

South Africa is in the process of developing food-based dietary guidelines (FBDG Working Group, 1998). In order to formulate sustainable guidelines regarding sugar consumption, it is important that these guidelines should address specific health problems in South Africa (FAO/WHO consultation, 1996). It is also important that the total diet be considered, since the incorporation of one food into the diet might have an effect on the nutrient composition of the total diet (FAO/WHO consultation, 1996). Overweight and obesity are known risk factors for CVD (Expert panel on the identification, evaluation, and treatment of overweight in adults, 1998). Sugar intake is often implicated as the villain in weight gain. In South Africa, even though many epidemiological studies could not demonstrate an association between added sugar intake and body mass index (BMI) (Bolton-Smith & Woodward, 1994; Fehily *et al.*, 1984; Lewis *et al.*, 1992; Tucker & Kano, 1992), the avoidance or restriction thereof is often recommended in weight loss programmes (Hanekom & Oosthuizen, 1997). Decreasing sugar intake might even increase weight gain due to the fat-sugar see-saw effect where decreased sugar consumption is associated with increased fat consumption (Bolton-Smith & Woodward,

1994; Baghurst *et al.*, 1992; Gibney *et al.*, 1989; Gibson, 1993; Kirk & Ruxton, 1997; Lewis *et al.*, 1992).

Sugar is also often implicated as "empty calories" not contributing to micronutrient intake. It is important to consider the possibility that consumption of sugar might have a nutrient diluting effect that might decrease important micro and macronutrients. With regard to CVD, fibre (Tables 2 and 3), and some anti-oxidants (vitamin E and ascorbic acid) (Stampfer & Rimm, 1995) which play important roles in the CHD risk reducing diet, may be decreased. The inverse association between added sugar and fat consumption has been well documented (Baghurst *et al.*, 1992; Gibney *et al.*, 1989). However, only a few studies have examined the effect of added sugar consumption on micronutrient and fibre intakes and these reported inconsistent patterns. It was therefore decided to examine the relationship between added sugar consumption and BMI and the effect of added sugar consumption on nutrient intake patterns in white South African men and women who participated in the VIGHOR study.

Recommendations following from this study could possibly be used in the formulation of guidelines regarding sugar consumption by South Africans. It might also be considered by the food industry for food labelling purposes and in the development of new, more acceptable products for CVD risk reducing diets and weight loss programmes. It may further be used by health professionals in dietary recommendations to the public.

1.4.3 Lecithin and serum lipoproteins, plasma fibrinogen and MPC levels

Most of the commercial lecithin brands claim hypocholesterolaemic effects and are therefore often prescribed by health practitioners for treatment of increased lipoprotein levels. The assumption that lecithin lowers lipoprotein levels is based on old (1940s to the early 1980s) studies which were poorly designed and which reported inconsistent results (see Introduction of Chapter 5).

The effects of lecithin on plasma fibrinogen and MPC levels have never been examined. Because plasma fibrinogen is positively associated with total cholesterol (TC), LDL-C and TG and negatively associated with HDL-C (Ernst & Resch, 1993), it is hypothesised that changes in lipoprotein levels that might be brought about by lecithin supplementation may also affect

plasma fibrinogen levels. It was decided to examine the independent hypocholesterolaemic effects of lecithin and to test the above hypothesis in a controlled study.

Intake of lecithin provides extra energy. It is a rich source of linoleic acid (on average 60-65%) (Knuiman *et al.*, 1989). Therefore, lecithin-specific effects will only be detected by providing equivalent amounts of fatty acids from another food source during a control period or to a control group to balance the effects of the fatty acids in the lecithin ingested by an experimental group (Knuiman *et al.*, 1989).

In this study the effects of a new, pure (96% phospholipids) soya bean lecithin product on serum lipoprotein, plasma fibrinogen and MPC levels will be examined by using a double-blind study design in which the extra energy and linoleic acid intake provided by lecithin are controlled by using sunflower oil.

Results from this study might confirm the hypocholesterolaemic claims already made on some lecithin brands, providing an additional means for lowering cholesterol levels. Labelling for health claims is underway to be legislated in South Africa. If no beneficial effects of lecithin were proven, the claim of hypocholesterolaemic effects on lecithin products might be forbidden in future.

1.4.4 High fibre *vs* low fibre meals and postprandial serum lipoprotein and plasma $FVII_c$ responses

The postprandial state can be an atherogenic state, since remnant particles formed from dietary-derived lipids are atherogenic (Zilversmit, 1979). Although the greatest part of the day is spent in this state, data on the effect of diet on postprandial lipoproteins are limited, whereas for the fasting state, a large body of literature is available. A few studies have examined the effect of soluble dietary fibre (especially oat bran) on postprandial lipoproteins, with inconsistent results (Cara *et al.*, 1992; Dubois *et al.*,1995; Dubois *et al.*, 1996; Redard *et al.*, 1990). In the one study that could be found which examined the effect of wheat bran, lower postprandial serum TG and chylomicron TG responses were reported compared to those after a test meal.

The activation of factor VII might be one of the mechanisms through which the increased presence of TG-rich lipoproteins in the circulation can influence atherogenesis. Studies which reported positive associations between fat intake and $FVII_c$ used very large amounts of fat, for example 1g fat/1 kg body weight (Freese & Mutanen, 1995; Salomma *et al.*, 1993) or 70-120g fat (Larson *et al.*, 1997; Oakley *et al.*, 1998; Sanders *et al.*, 1996).

It was therefore decided to examine the effects of high fibre *vs* low fibre test meals that provided realistic physiological amounts of fat usually eaten in a meal (30.5g fat) on postprandial serum lipoprotein and $FVII_c$ responses.

Results from this study might be used by the food industry in the development of new, more acceptable health foods or functional foods. Functional foods are defined as formulated foods or beverages that provide physiological benefit beyond basic nutrition, in order to maintain or improve health (Williams 1998). It might also be used on food labels for health claims and it might be used by dieticians in the primary prevention and management of CVD.

1.4.5 Extruded dry beans and serum lipoproteins and plasma haemostatic factors

The inclusion of dry beans in the hypocholesterolaemic diet is encouraged, because it is a rich and economical source of plant protein, complex carbohydrates, soluble and insoluble dietary fibre components and a variety of minerals and vitamins. It is also low in energy, fat and sodium (Vorster & Venter, 1994b). Cholesterol-lowering effects of dry beans have been reported (Anderson *et al.*, 1984; Anderson *et al.*, 1990), although the results are inconsistent. In the studies of Cobiac *et al.* (1990) and Mackay and Ball (1992), dry beans had no effect on lipoprotein concentrations. The effect of dry beans on haemostatic variables have not been studied. Because of its high content of soluble fibre, it may have beneficial effects on plasma fibrinogen (Koepp & Hegewisch 1981; Venter *et al.*, 1990; Venter *et al.*, 1991) and PAI-1 (Djoussé *et al.*, 1998; Landin *et al.*, 1992; Sundell & Rånby, 1993) levels.

Extrusion of dry beans is a relatively new process. It provides an economically and convenient alternative to cooking and canning and provides a variety of options for ingesting this health food.

In this randomised controlled cross-over study the effects of the inclusion of extruded dry beans, as a new functional food, in the diet on serum lipoproteins, plasma fibrinogen, plasma viscosity and PAI-1 levels were therefore examined.

Results from this study might be used by the food industry in the development of alternative and acceptable options for ingesting dry beans as part of the hypocholesterolaemic diet. If beneficial effects of extruded dry beans on these CVD risk factors could be indicated, it might lead to health claims with regard to this product and to the promotion of dry beans as part of the healthy diet.

2. AIMS AND OBJECTIVES

The aims and objectives of this thesis were:

2.1 Main aim: To describe the mean alcohol consumption and its association with risk factors for CHD in randomly selected white men and women from the industrialised communities of Witbank and Vanderbijlpark who participated in the VIGHOR project.

Objectives:

1) To examine the mean daily alcohol intake of men and women, stratified for age;

2) to examine the relationship between alcohol intake and:
   - blood pressure;
   - serum lipoproteins: TC, HDL-C, LDL-C, TG; and
   - gamma-glutamyl transferase (proxy for alcohol intake to verify the accuracy of the measurement of alcohol intake),

   controlling for potential confounding factors such as age, body mass index (BMI), smoking, physical activity, education and income.

2.2 Main aim: To investigate dietary composition and BMI at different levels of added sugar consumption in men and women from the industrialised communities of Witbank and Vanderbijlpark who participated in the VIGHOR project.

Objectives:

To investigate the relationship between added sugar consumption and:

- BMI, controlling for age, physical activity, smoking and alcohol consumption;
- macronutrient intake patterns, controlling for BMI, age, physical activity, smoking, alcohol consumption and energy intake; and
- micronutrient intake patterns.

2.3 Main aim: To investigate the effects of a new, pure soya bean lecithin product on serum lipoproteins and haemostatic factors in hyperlipidaemic men in a double-blind controlled study.

Objectives:

1) To monitor compliance by analysing the fatty acid composition of total plasma lipids;

2) to investigate the effects of lecithin on:
   - Serum lipoproteins: TC, TG, HDL-C, LDL-C, apoprotein (apo) A, apoB and Lipoprotein (a) (Lp(a));
   - haemostatic factors: plasma fibrinogen and MPC; and
   - haematocrit and haemoglobin.

2.4 Main aim: To examine the effects of a high fibre *vs* a low fibre meal on postprandial serum lipoprotein and plasma $FVII_c$ responses.

Objectives:

To examine the effects of a high fibre *vs* a low fibre meal on postprandial responses of:

- Serum lipoproteins: TG, TC and HDL-C;
- plasma retinyl palmitate (marker for chylomicrons and their remnants);
- serum insulin; and
- plasma $FVII_c$.

2.5 Main aim: To examine the effects of the inclusion of extruded dry beans in the diet on serum lipoproteins and haemostatic factors in hyperlipidaemic men.

Objectives:

1) To monitor dietary changes and compliance by estimating food and nutrient intakes;

2) to examine the effects of the inclusion of extruded dry beans in the diet on:
   - Serum lipoproteins: TC, TG, HDL-C, LDL-C, apoA, apoB and Lp(a);
   - haemostatic factors: plasma fibrinogen, PAI-1 activity and plasma viscosity; and
   - haematocrit and haemoglobin.

3. STRUCTURE OF THIS THESIS

This thesis consists of three published papers and two manuscripts submitted for publication. Following this introductory chapter, Chapter 2 gives an overview of the lipid and lipoprotein metabolism, haemostatic processes and the pathogenesis of atherosclerosis. This chapter provide the background necessary for the interpretation of the data from the papers in this thesis. Chapter 3 addresses alcohol consumption and its relationship with risk factors for CHD. The effects of different levels of added sugar consumption on dietary composition and BMI are discussed in Chapter 4. Chapter 5 examines the effects of lecithin on serum lipoprotein, plasma fibrinogen and MPC levels in hyperlipidaemic men. The postprandial serum lipoprotein and $FVII_c$ responses after consumption of high fibre *vs* low fibre muffins are compared in Chapter 6. The effects of extruded dry beans on serum lipoproteins and haemostatic factors are examined in hyperlipidaemic men in Chapter 7. Chapters 3, 4 and 5 have been published, while chapters 6 and 7 were submitted for publication. In Chapter 8 a general discussion and summary of all the results are provided, recommendations are made and conclusions are drawn. This thesis does not contain a separate literature survey because the appropriate and relevant literature backgrounds are discussed in each separate publication. The relevant references are provided at the end of each chapter according to the authors instructions of the specific journal in which the papers were published or submitted for publication. The relevant references used in the unpublished chapters 1, 2 and 8 are provided

according to the mandatory style stipulated by the Potchefstroom University for Christian Higher Education. The technical style used in the unpublished chapters are uniform, but differs in the other chapters according to the authors instructions of the specific journals.

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CHAPTER 2

CARDIOVASCULAR DISEASE: LIPIDS, LIPOPROTEINS AND THE HAEMOSTATIC SYSTEM

1. INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of mortality and morbidity in South Africa (Bradshaw *et al.*, 1995). Lipids, lipoproteins and some factors involved in coagulation and fibrinolyses may contribute to the risk for CVD and these factors may be modified by dietary intervention (Shrapnel *et al.*, 1992; Vorster *et al.*, 1997). In order to interpret results from these types of studies, it is important to understand the processes involved in the metabolism of these factors and how they can contribute to the development of CVD.

Although several authors have reviewed the lipid and lipoprotein metabolism and haemostatic processes, small discrepancies do occur. There is also a lack of reviews that provide a comprehensive overview of the above processes and the roles that both play in the pathogenesis of atherosclerosis. In this section the characteristics, functions and metabolism of lipids and lipoproteins as well as haemostatic factors will be explained. The role that these two systems play in the pathogenesis of atherosclerosis will also be discussed. Information from the most recent review articles and textbooks will be synthesised and summarised to give a relatively simplified and comprehensive overview of the most important and corresponding processes involved. Firstly, definitions for terminology referring to CVD, will be given.

2. TERMINOLOGY: CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD): A general term that refers to diseases of the heart (cardio-) and blood vessels (-vascular) that carry blood to all the tissues in the body (Brink *et al.*, 1979:230-231; Martin, 1993:273, 627).

Coronary heart disease (CHD)/Ischaemic heart disease: A general term that refers to diseases of the heart. The main cause is atherosclerosis that causes occlusion of the arteries that carry blood to the heart muscle causing the heart to suffer ischaemia and damage (Whitney *et al.*, 1998:164).

Myocardial infarction: Damage to a segment of the heart because of ischaemia of the myocardium as a result of an obstruction in the arteries (Martin, 1993:363).

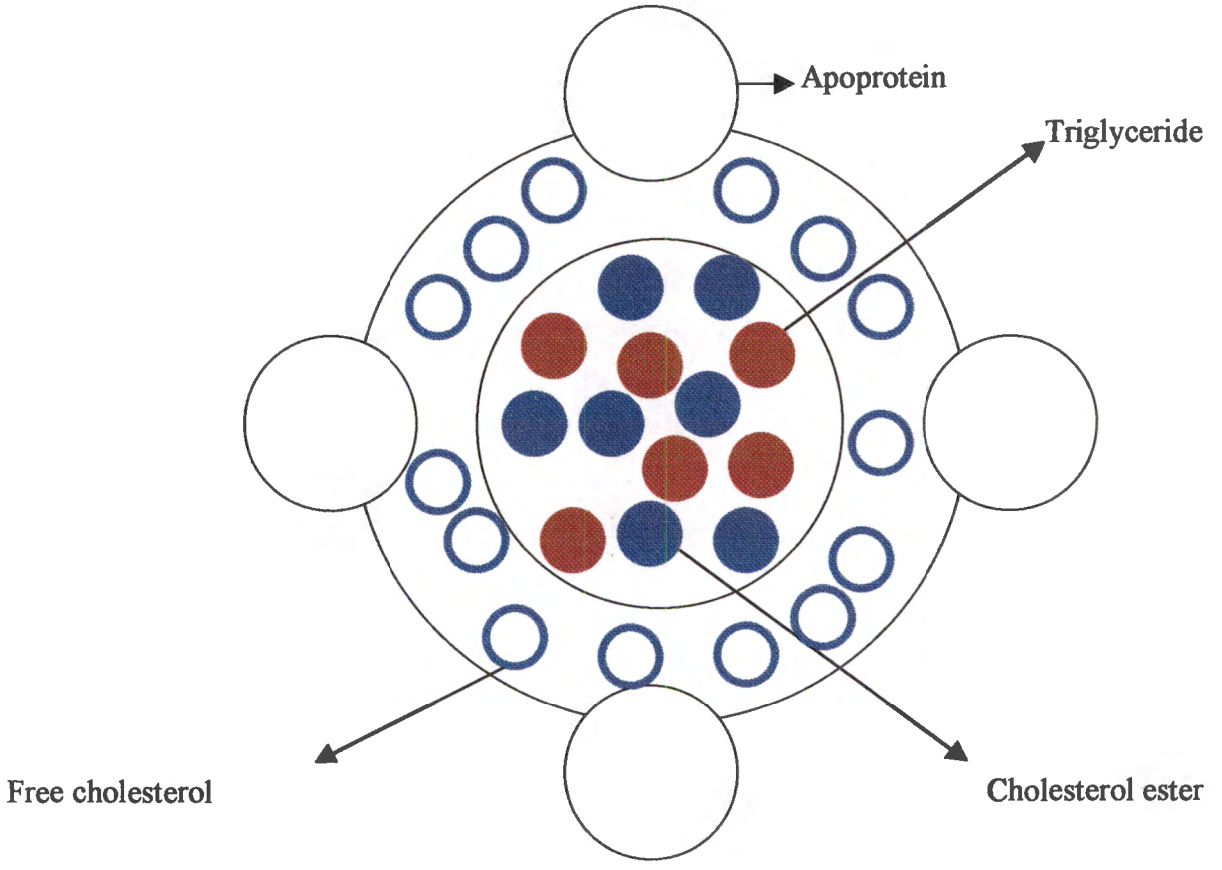

Figure 1 **Structure of a lipoprotein particle** (adapted from Griffith *et al.*, 1996:13.38).

Angina pectoris: A tightness in the centre of the chest that radiates through to the back and shoulder or down one or both arms or into the neck and jaw (Pumphrey, 1996:5.35) because of insufficient blood flow to the heart (Martin, 1993:26).

Cerebrovascular disease/stroke: A term that refers to diseases of the brain. Stroke can be caused by either insufficient blood flow to the brain because of atherosclerosis or thrombus formation (ischaemic stroke) or by rupture of a cerebral artery because of hypertension (haemorrhagic stroke) (Martin, 1993:60, 520-521).

Atherosclerosis: The development of fatty plaque within the intima and media of medium and large arteries causing thickening of the arterial wall and obstruction of blood flow to the heart and other tissue (Lichtenstein, 1996:430).

Thrombosis: The formation of a thrombus or blood clot, which may obstruct blood flow to the heart and other tissue (Whitney *et al.*, 1998:883).

3. LIPIDS AND LIPOPROTEINS

3.1 Introduction

Lipids, lipoproteins and apoproteins are important since they are associated with increased risk of CHD. Total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) (Adult Treatment Panel II, 1994), triglycerides (TG) (Castelli, 1996), apoprotein (apo) B (Brown *et al.*, 1990), chylomicron remnants (Zilversmit, 1995) and lipoprotein (a) (Lp(a)) (Lawn, 1992) are positively associated with CHD. High density lipoprotein cholesterol (HDL-C) (Castelli, 1990) and apoA (Anderson & Akanji, 1994) are negatively associated with CHD.

In this section the characteristics, functions and metabolism of the different lipids, lipoproteins and apoproteins will be discussed.

Since lipids (major lipids = cholesterol and TG) are not soluble in water, they associate with specific proteins, the apoproteins, to form lipoprotein complexes that are soluble in the blood. Lipoproteins in the circulation are thus responsible for the transport of the major lipids. The structure of a lipoprotein is illustrated in Figure 1. The insoluble cholesterol esters (CE) and TG form the core of the lipoprotein particle and are surrounded by the more polar substances, free cholesterol (FC) and phospholipids (PL), while the apoproteins extend over the surface. There are five classes of lipoproteins and five major groups of apoproteins that play important

and distinct roles in the lipid and lipoprotein metabolism. These will be further discussed, based on recent reviews by Griffith *et al.* (1996:13.38), Mann & Skeaff (1998:38-42) and Wolmarans (1997). The desirable levels for lipids and lipoproteins are summarised in Table 1.

Table 1 The desirable levels for lipids, lipoproteins, and some of the apoproteins

Lipoprotein	Desirable level	Reference
TC	<5.2 (mmol/L)	Adult Treatment Panel II (1994)
LDL-C	<3.4 (mmol/L)	Adult Treatment Panel II (1994)
HDL-C	>1.0 (mmol/L)	Gotto *et al.* (1995:27)
TG	<2.3 (mmol/L)	Adult Treatment Panel II (1994)
Lp (a)	<300 (mg/L)	Harris (1997)
ApoA	>1.2 (g/L)	Adult Treatment Panel II (1994)
ApoB	<1.2 (g/L)	Adult Treatment Panel II (1994)

TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; TG: Triglyceride; Lp(a): Lipoprotein (a); Apo: Apoprotein

3.2 Characteristics and functions of lipoproteins

The lipoproteins differ in density, which makes separation by density gradient ultracentrifugation possible. They also differ in size, lipid and apoprotein content and function. The characteristics and functions of the lipoproteins are summarised in Table 2 and their role in the lipid and lipoprotein metabolism is illustrated in Figure 2.

When a laboratory measures fasting serum lipids, the majority of the TC concentration consists of LDL-C with a 20-30% contribution from HDL-C. The TG concentration largely reflects the circulating number of very low density lipoprotein (VLDL) particles, since chylomicrons are not present in the fasting state (Gale & Anderson, 1996:855)

Table 2 Characteristics and functions of lipoproteins (summarised by Mann & Skeaff, 1998:40; Wolmarans 1997:7)

Lipoprotein	Density (g/mL)	Percentage of total lipids (% weight)				Major apoproteins (composition {weight %})	Origin	Function
		TG	PL	CE	FC			
Chylomicron	<0.95	88	8	3	1	As, B-48, (Cs and E transferred from HDL), (2%)	Intestine	Transport of dietary lipids from intestine to peripheral tissues
Chylomicron remnant	<0.95	?	?	?	?	B-48, E (As and Cs transferred to HDL)	Formed from chylomicrons	Delivery of cholesterol to liver
VLDL	0.95-1.006	56	20	15	8	B-100, (Cs and E transferred from HDL) (10%)	Liver	Transport of lipids from liver to peripheral tissues
IDL	1.006-1.019	29	26	34	9	B-100, E (Cs transferred to HDL) (11%)	Formed from VLDL	Precursor of LDL or transport of lipids to liver
LDL	1.019-1.063	13	28	48	10	B-100 (21%)	Formed from VLDL and IDL	Transport of cholesterol to peripheral tissues and liver
Lp(a)	1.05-1.12	11	29	48	11	B-100, apo(a) (31%)	Liver	Unknown
HDL_2	1.063-1.125	16	43	31	10	As, Cs, E (33%)	See 3.5.3 - formation of HDL	Reverse cholesterol transport
HDL_3	1.125-1.210	13	46	29	6	As, Cs, E (43%)	See 3.5.3 - formation of HDL	Reverse cholesterol transport

VLDL: Very low density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low density lipoprotein; Lp(a): Lipoprotein (a); HDL: High density lipoprotein; TG: Triglyceride; PL: Phospholipids; CE: Cholesterol esters; FC: Free cholesterol

3.3 Lipoprotein (a)

Lp(a) is a genetical variant of LDL. It is identical to LDL, except that it has an additional protein component, apo(a), that becomes linked to the apoB100 component of LDL via a disulfide bridge (reviewed by Harris, 1997).

The apo(a) structure resembles that of plasminogen, the precursor to plasmin, responsible for dissolving fibrin clots. Moreover, the gene for apo(a) is located on chromosome 6 and is adjacent to the gene for plasminogen. This feature of apo(a) may explain some of Lp(a)'s pathogenicity, because Lp(a) competes with plasminogen for binding sites on fibrin and may thereby decrease fibrinolysis (reviewed by Harris, 1997).

3.4 Functions and characteristics of apoproteins

Apoproteins play a very important role in the lipid and lipoprotein metabolism. The principal functions of the major apoproteins are summarised in Table 3 and their role in the lipid and lipoprotein metabolism is illustrated in Figure 2.

An individual's apoprotein profile is genetically determined and variants are being recognised that may result in specific metabolic disorders (Gurr, 1993:95).

Table 3 **The principal functions of the major apoproteins** (summarised by Griffith *et al.*, 1996:13.39)

Apoprotein	Principal origin	Principal function
AI	Liver, intestine	LCAT activator
AII	Liver, intestine	Structural protein in HDL
AIV	Liver, intestine	Non-specific LCAT cofactor
B48	Intestine	Mediates chylomicron formation and secretion by enterocytes
B100	Liver	Mediates VLDL formation, ligand for LDL receptor
CI	Liver	Inhibitor of chylomicron uptake
CII	Liver	LPL activator
CIII	Liver	Inhibitor of chylomicron? Inhibitor of LPL
E	Liver	Ligand for chylomicron and LDL receptor

LCAT: Lecithin : cholesterol acyltransferase; LPL: Lipoprotein lipase; VLDL: Very low density lipoprotein; LDL: Low density lipoprotein; HDL: High density lipoprotein

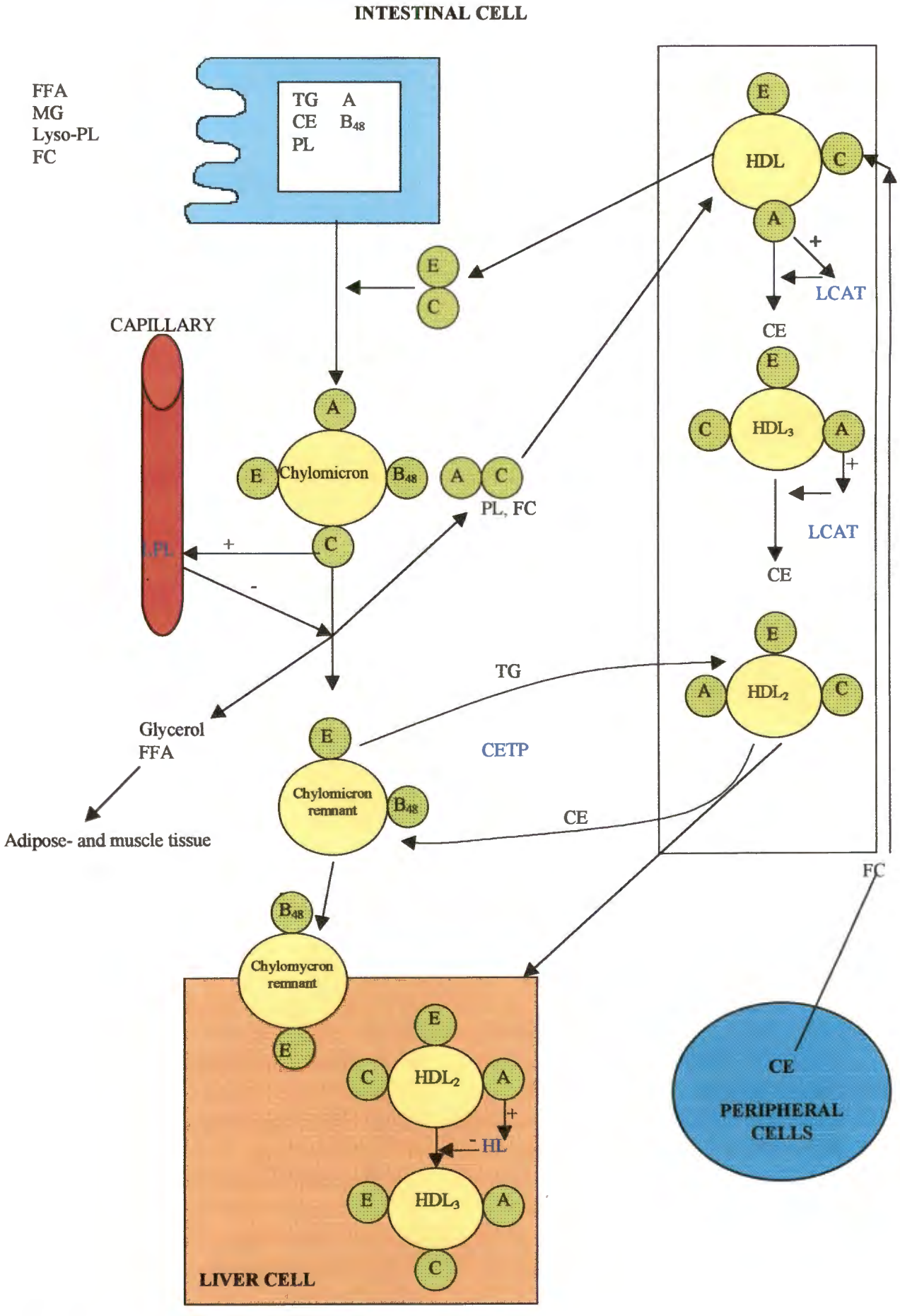

Figure 2a (figure description on page 32)

3.5 Lipid and lipoprotein metabolism

3.5.1 The exogenous pathway

The exogenous pathway is illustrated in Figure 2a. Dietary fats (mainly TG, CE and PL) entering the intestine are emulsified by bile to make them accessible for hydrolysis by digestive enzymes. Small units of digested fat (glycerol and fatty acids with chain length <12 carbon atoms) enter the portal vein system directly by diffusing across the enterocytes of the small intestine. They are transported in blood bound to albumin. Larger units (mainly monoglycerides {MG}, free fatty acids {FFA}, FC, lysophospholipids {Lyso-PL}) merge into spherical complexes known as micelles and diffuse into the enterocytes where they are re-synthesised. The newly synthesised CE, PL and TG are packed into chylomicrons, containing apoA and apoB48. These are released into the lymphatic system until they reach the circulation at the thoracic duct near the heart (reviewed by Mann & Skeaff, 1998:37; Whitney *et al.*, 1998:154-158).

Upon entering the bloodstream, chylomicrons pick up apoC and apoE from HDL. The chylomicrons pass through the lungs and ventricles of the heart with little modification and then rapidly enter the capillaries of the skeletal muscles, heart, mammary glands and adipose tissue. ApoCII then activates lipoprotein lipase (LPL) on the walls of the capillaries causing TG to be broken down to glycerol and FFA (70-80% of the TG in chylomicrons are degraded). FFA is taken up primarily by adipose tissue for storage and muscle tissue for energy. During breakdown of TG, some FC and PL, along with apoA and apoC, pinch off to form HDL. The resulting chylomicron remnant, containing proportionately less TG and more cholesterol, binds to receptors on the liver cell through apoE, which serves as a ligand, and are removed from the circulation. In the liver, cholesterol is used for membrane or new lipoprotein biosynthesis, or is converted into bile acids (reviewed by Mann & Skeaff, 1998:39).

Figure 2a The exogenous pathway of lipid and lipoprotein metabolism (adapted from Barakat *et al.*, 1996; Griffith *et al.*, 1996:13.39; Gurr, 1993:95; Mann and Skeaff, 1998:39)
FFA: Free fatty acids; MG: Monoglycerides; Lyso-PL: Lysophospholipids; FC: Free cholesterol; TG: Triglycerides; CE: Cholesterol esters; PL: Phospholipids; A, B, C, E: Apoproteins; LPL: Lipoprotein lipase; HDL: High density lipoprotein; LCAT: Lecithin : cholesterol acyltransferase; HL: Hepatic lipase; CETP: Cholesterol ester transport protein; +: Activate; -: Hydrolyse.

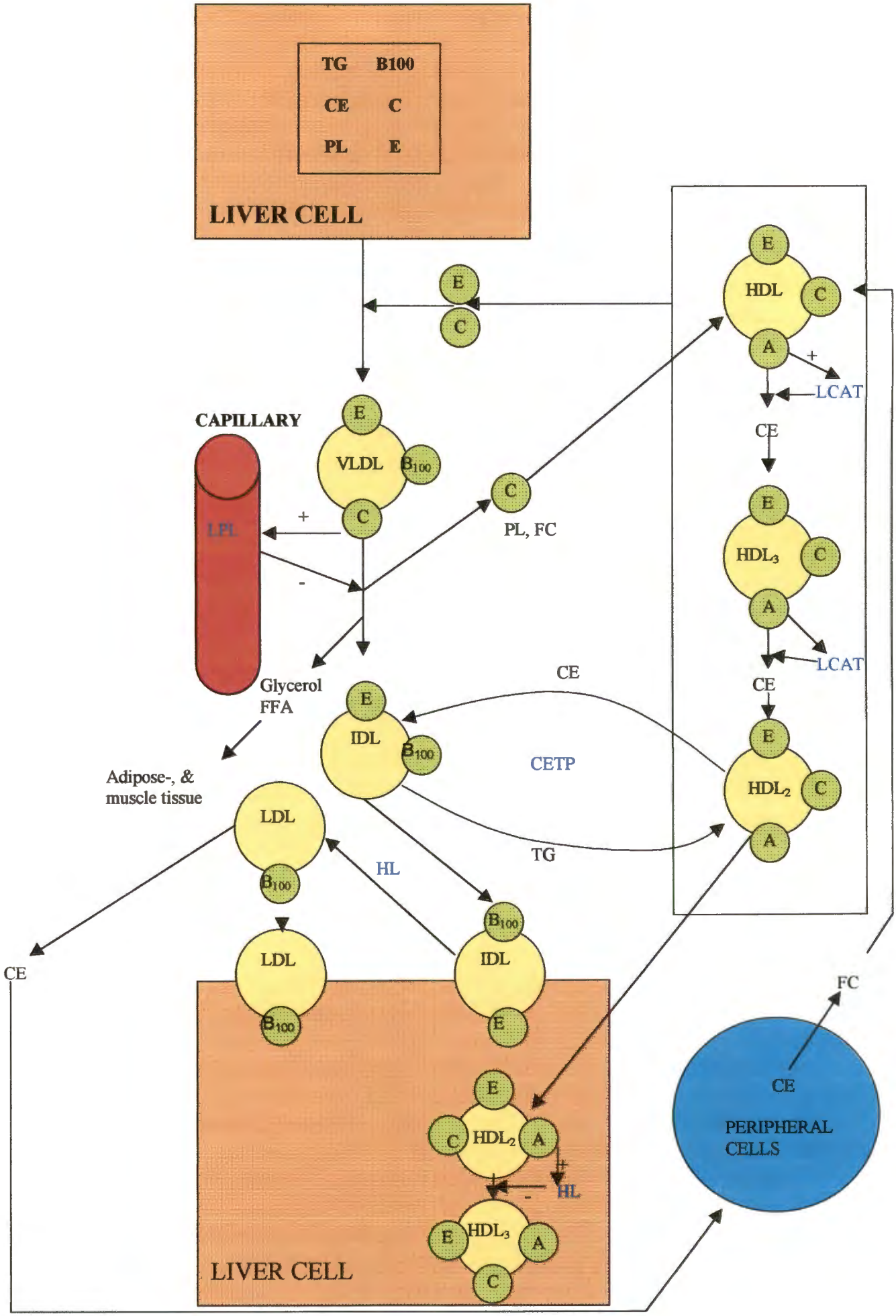

Figure 2b (figure description on page 34)

3.5.2 The endogenous pathway

The endogenous pathway is illustrated in Figure 2b. Lipids synthesised in the liver and those delivered to the liver by chylomicron remnants are packaged into VLDL, containing apoB100, apoC and apoE, and secreted into the blood. VLDL picks up apoC and apoE from HDL. LPL, activated by apoCII, hydrolyses TG in VLDL, resulting in the formation of intermediate density lipoprotein (IDL). The FFA are transferred to adipose and muscle tissue. During breakdown of TG, some FC and PL along with apoC, pinch off to form HDL. IDL binds to receptors on the liver cells through apoE, that serves as a ligand for the receptor. Once bound, IDL can be catabolised, or undergo further TG removal by the enzyme hepatic lipase (HL), producing LDL particles. Cholesterol is delivered to peripheral and liver cells when LDL binds to LDL receptors through apoB100, that serves as a ligand for the receptor (reviewed by Gale & Anderson, 1996:854-855; Gurr, 1993:96; Mann & Skeaff, 1998:39). The discharge of cholesterol to peripheral cells can also occur by passive endocytosis (summarised by Gurr, 1993:96).

3.5.3 Reverse cholesterol transport

The formation of HDL and its role in reverse cholesterol transport is illustrated in figures 2a and 2b.

Figure 2b The endogenous pathway of lipid and lipoprotein metabolism (adapted from Barakat *et al.*, 1996; Griffith *et al.*, 1996:13.39; Gurr, 1993:95; Mann and Skeaff, 1998:39)
TG: Triglycerides; CE: Cholesterol esters; PL: Phospholipids; FC: Free cholesterol; A, B, C, E: Apoproteins; LPL: Lipoprotein lipase; HDL: High density lipoprotein; VLDL: Very low density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low density lipoprotein; LCAT: Lecithin : cholesterol acyltransferase; HL: Hepatic lipase; CETP: Cholesterol ester transport protein; +: Activate; -: Hydrolyse.

Formation of HDL

HDL is secreted by die intestine and liver as nascent HDL and has a spherical form. During hydrolysis, by LPL, of TG in chylomicrons and VLDL, nascent HDL is provided with apoA, and apoC, PL and FC and change to a more discoidal form. The PL and FC serves as substrates for the formation of CE through lecithin : cholesterol acyltransferase (LCAT), that catalyses the transfer of a fatty acid from position *sn*-2 of phosphatidylcholine to cholesterol to form CE. LCAT circulates with the HDL particles and the reaction therefore occurs on the surface of the HDL particle. The HDL particle gradually gets more enriched with CE and HDL_3 and then HDL_2 are formed. Total HDL-C concentration that is measured in the laboratory mainly resembles HDL_2, the more CE enriched form of HDL (reviewed by Barakat *et al.*, 1996; Fossati & Romon-Rousseaux, 1987).

Reverse cholesterol transport

The liver is the only site of cholesterol excretion and HDL is involved in returning cholesterol from peripheral tissues to the liver. HDL particles are able to take up cholesterol from the arterial wall. There is evidence to suggest that cells loaded with CE express receptors for HDL (apoAI receptors), which activate the hydrolysis of the fatty acids from CE and promote efflux of FC from cells. FC is then esterified by LCAT through activation by apoAI. The CE formed on HDL may transfer to lipoproteins of lower density (chylomicron remnants, IDL and VLDL) through a cholesterol ester transport protein (CETP) where HDL donates CE in exchange for TG from these lipoproteins. The CE is then returned to the liver via these lipoproteins. HDL_2 is degraded in the liver when the TG is removed from the HDL particle by HL. The particles that exit from the liver (HDL_3) have a decreased neutral lipid content, are smaller and denser, and can accept more CE (Barakat *et al.*, 1996; Griffith *et al.*, 1996:13.39; Roche & Gibney, 1995).

Alternatively the CE may be returned to the liver directly in the HDL particle by binding to the LDL receptor through apoE, that serves as a ligand for the receptor. Most of the CE are, however, returned to the liver via chylomicron remnants and IDL (summarised by Griffith *et al.*, 1996:13.39; Sethi *et al.*, 1993).

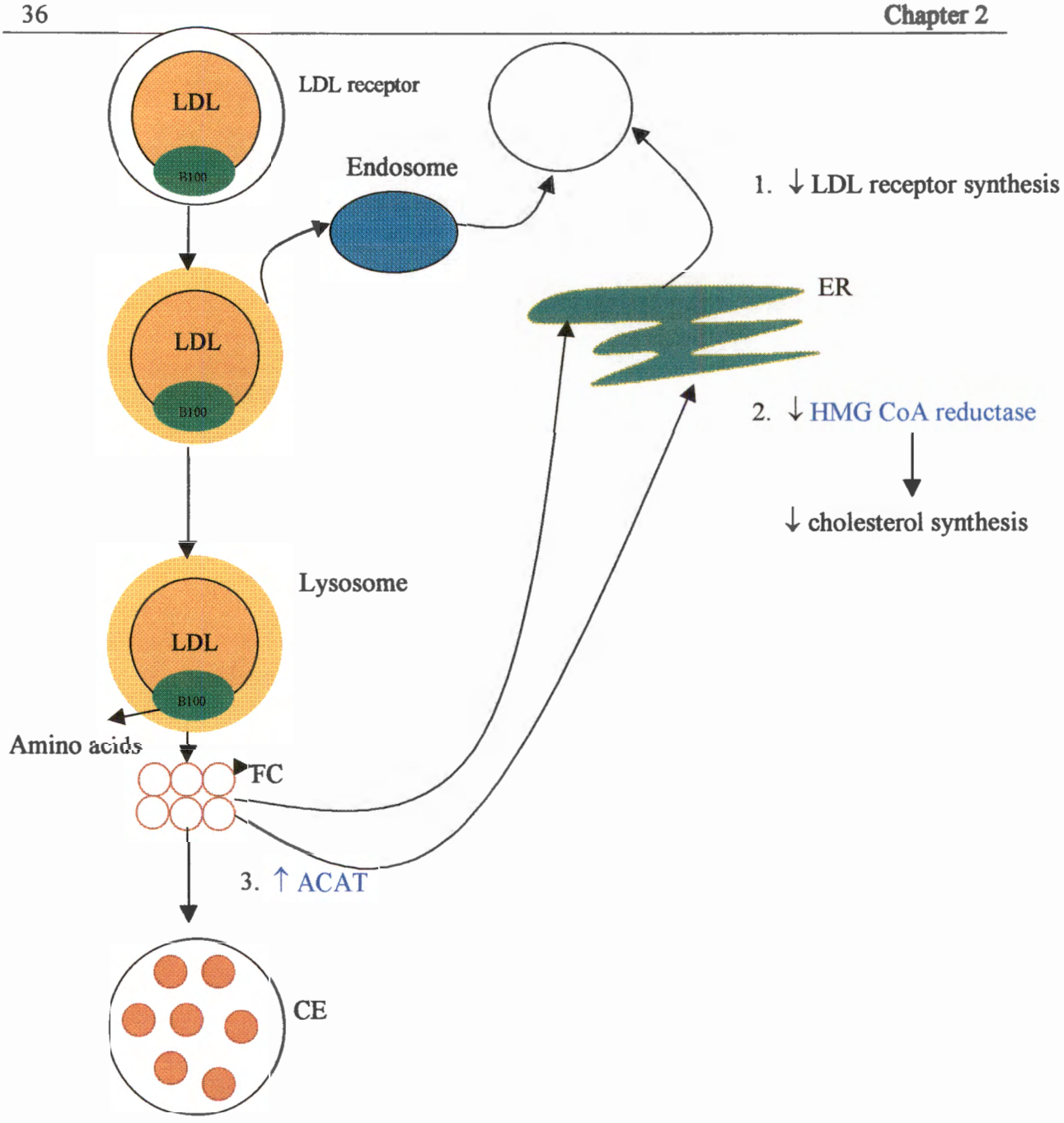

Figure 3 **Receptor mediated uptake of cholesterol and cellular cholesterol homeostasis** (adapted from Goldstein and Brown, 1989:1226)
ER: Endoplasmic reticulum; ACAT: Acyl CoA:cholesterol acyltransferase: HMG CoA: Hydroxymethyl-glutaryl-CoA; LDL: Low density lipoprotein; B: Apoprotein B: FC: Free cholesterol; CE: Cholesterol ester.

3.5.4 Receptor mediated uptake of cholesterol and cellular cholesterol homeostasis

The studies by Brown and Goldstein in the 1970s and 1980s disclosed the existence of the cell surface LDL receptor, one of the key elements in cellular cholesterol homeostasis.

Figure 3 illustrates the receptor mediated uptake of cholesterol and cellular cholesterol homeostasis. LDL particles are the main carriers of cholesterol, and deliver it to the liver and peripheral cells via the LDL receptor. Strategically located on the surfaces of cells, lying within coated pits, these receptors bind LDL with high affinity through apoB100, that serves as the ligand for the LDL receptor. The lipoprotein-receptor complex is then carried into the cell by receptor-mediated endocytosis. The receptor and LDL are dissociated and the receptor is recycled to the cell surface. The LDL is delivered to a lysosome where it is hydrolysed by acid hydrolytic enzymes. The resulting FC crosses the lysosomal membrane and enters the cellular component, where it is used for the synthesis of membranes, bile acids, steroid hormones and as a regulator of intracellular cholesterol homeostasis (Goldstein & Brown, 1989:1226-1228).

The intracellular cholesterol homeostasis is a sophisticated system of feedback control to co-ordinate the intracellular and extracellular sources of cholesterol so as to maintain a constant level of cholesterol within the cell. The increase of FC in the cell causes the following:

1) Reduction of LDL receptor synthesis in the endoplasmic reticulum, preventing further entry of LDL and thereby protecting cells against an overaccumulation of cholesterol.

2) Suppression of the activity of hydroxymethyl-glutaryl-CoA (HMG CoA) reductase, the rate-controlling enzyme in endogenous cholesterol biosynthesis, thereby turning off cholesterol synthesis in the cell.

3) Activation of a cholesterol-esterifying enzyme, called acyl CoA:cholesterol acyltransferase (ACAT), to catalyse the storage of excess cholesterol as CE.

When LDL receptor function is inappropriately diminished as a result of genetic defects (for example familial hypercholesterolaemia) or in response to regulatory signals, cholesterol cannot be removed from the circulation and builds up in plasma and atherosclerosis ensues (Goldstein & Brown, 1989:1226-1228).

4. THE HAEMOSTATIC SYSTEM

Heamostasis means the ability to prevent or arrest the flow of blood from an injured vessel. The efficiency of this process depends on a complex interaction between the vessel wall, platelet aggregation, the coagulation system and the fibrinolytic system. Failure of any one of these four components can result in either haemorrhagic or thrombotic tendency (reviewed by Reilly & Cawley, 1996:11.8).

Many of the factors that play a role in the haemostatic process have been implicated in epidemiological studies to be risk factors for CHD. For some of these factors there are strong evidence in the literature regarding their association with CHD, namely fibrinogen, factor VII and plasminogen activator inhibitor 1 (PAI-1). The evidence for other factors, namely antithrombin III, tissue plasminogen activator (tPA) and d-dimer, is insufficient to draw conclusions at this stage (reviewed by Jerling, 1998:14-24).

In this section the four components of the heamostatic system, namely the vessel wall, platelet aggregation and the coagulation and fibrinolytic systems, will be discussed separately although it is important to bear in mind that their functions are interactive.

4.1 The vessel wall

Vessel wall endothelial cells possess both anti and prothrombotic properties. Its antithrombotic activities include synthesis and secretion of anticoagulant and profibrinolytic substances, namely:

- Prostacyclin (PGI): Inhibitor of platelet aggregation;
- protein C & S: Protein C is an anticoagulant by inhibiting active factor V and VIII and it induces fibrinolysis through inhibition of PAI-1. Protein S is a cofactor for protein C;
- thrombomodulin: Binds to thrombin which then activates protein C;
- tissue factor pathway inhibitor (TFPI): Anticoagulant by inhibiting the factor VII-tissue factor (TF) complex that catalyses the coagulation process;
- tissue plasminogen activator (tPA): Regulates the fibrinolytic system;
- urokinase plasminogen activator (uPA): Regulates the fibrinolytic system; and

- glycosaminoglycans (heparan sulphate): Inhibits smooth muscle cell migration and proliferation.

When the endothelial cells are injured or stimulated by substances such as thrombin or inflammatory and pathological agents, the endothelial cells acquire prothrombotic properties. The endothelial cells localise the formation of thrombin and procoagulant and antifibrinolytic substances that are synthesised and secreted, namely:

- Tissue factor (TF): Activates the extrinsic coagulation pathway by activating factor VII; and
- plasminogen activator inhibitor 1 (PAI-1): Inhibits the action of tPA.

(Reviewed by Jerling, 1998:12; Murphy, 1994:336; Reilly & Cawley, 1996:11.8-11.9; Vorster *et al.*, 1997).

4.2 Platelet aggregation

Platelet aggregation and the formation of a platelet plug is part of the haemostatic mechanism to prevent blood loss from injured vessels. Aggregating platelets participate in fibrin network formation, clot retraction and tissue repair. The role of platelets in the pathogenesis of atherosclerosis is illustrated in Figure 5.

Damaged endothelium exposes collagen. Platelets, circulating in the blood, adhere to the collagen through platelet membrane receptors. These receptors are glycoprotein Ia, which binds directly to collagen, and glycoprotein Ib, which binds to collagen via Von Willebrand factor (VIII:vWF). Following adhesion, the platelets undergo a change in shape from a disc to spherical, spread along the subendothelium and release their granule contents namely, adenosine 5`-diphosphate (ADP), serotonin, platelet-derived growth factor (PDGF), platelet factor 4 (PF4), β-thromboglobulin, fibrinogen, VIII:vWF and other factors. The release of ADP leads to exposure of a fibrinogen receptor, the glycoprotein IIb-IIIa complex (GPIIb-IIIa), on surfaces of adherent platelets. Binding of fibrinogen to the GPIIb-IIIa enables interplatelet aggregation. Further platelet membrane receptors are exposed during aggregation, providing a surface for the interaction of coagulation factors. This platelet activity is referred to as platelet factor 3 (PF3).

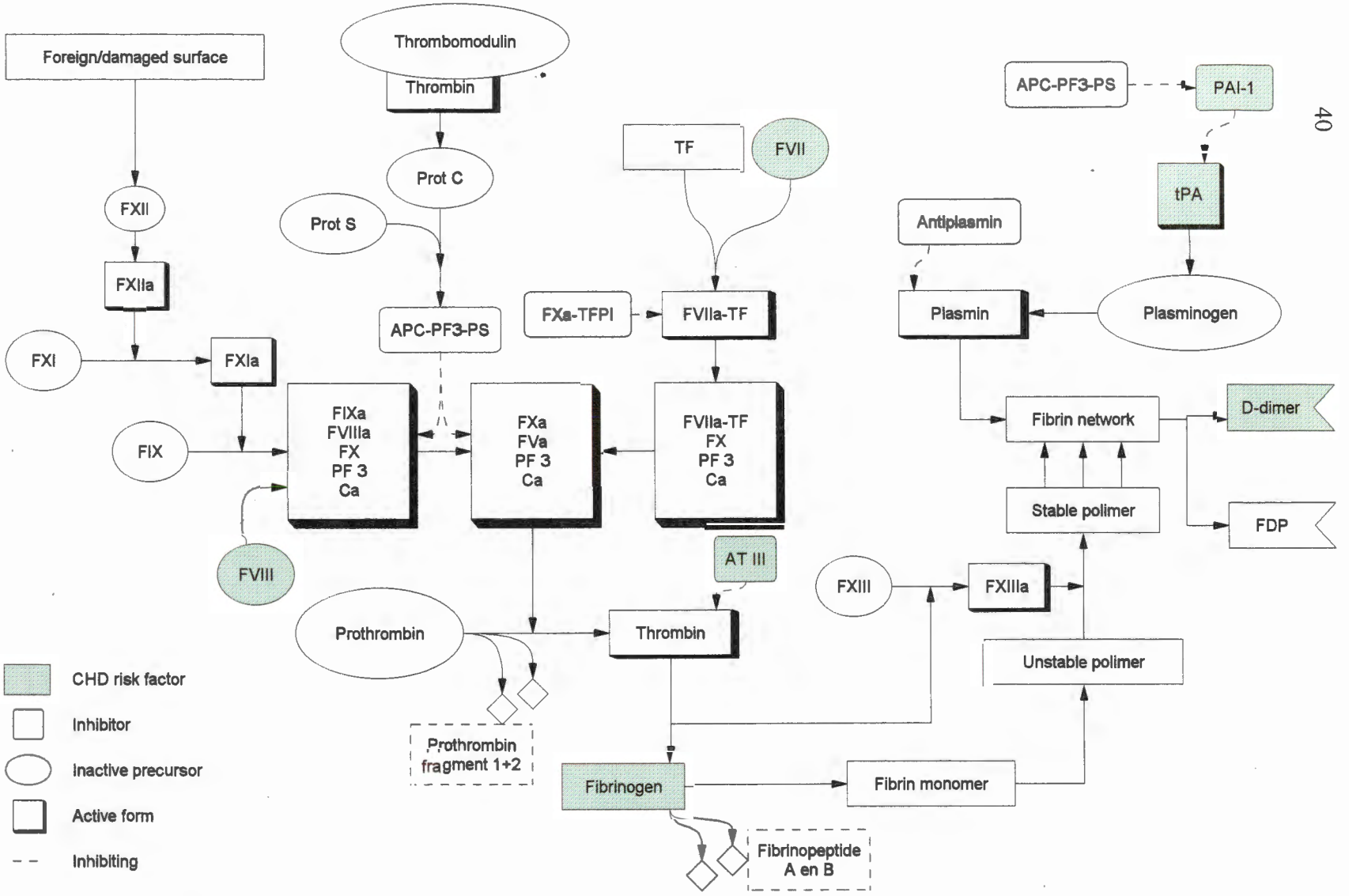

Figure 4 (figure description on page 41)

Contact of platelets with collagen leads to the liberation of arachidonic acid from membrane PL that is converted by cyclo-oxygenase to thromboxane A (TXA), a powerful platelet aggregating agent and vasoconstrictor. PGI synthesised in vascular endothelial cells opposes the actions of TXA.

The presence of thrombin encourages fusion of platelets and the formation of cross-linked fibrin strands that stabilise the platelet plug by binding platelets and red cells. Increased platelet aggregation plays an important role in the development of atherosclerosis and thrombosis (Reviewed by Jerling, 1998:11; Murphy, 1994:336; Reilly & Cawley, 1996:11.9; Vorster *et al.*, 1997).

4.3 Coagulation system

Coagulation is an autocatalytic, self-limiting process that involves a sequential "cascade" of enzymatic reactions leading to the conversion of soluble fibrinogen into an insoluble cross-linked fibrin clot. The coagulation system is illustrated in Figure 4 and its role in the pathogenesis of atherosclerosis in Figure 5. The system can be activated by one of two pathways, namely the intrinsic or the extrinsic pathway:

- *Intrinsic pathway:* Factor XII is activated when the blood comes in direct contact with foreign or damaged surfaces.
- *Extrinsic pathway:* Thromboplastin (TF), released from damaged cells, binds to factor VII, which is then activated.

The resulting activated factor XII and factor VII-TF complex act as catalysts for a "cascade" of reactions that ultimately lead to the formation of thrombin. The principle of this "cascade" of reactions is that an activated clotting factor (proteinase) activates another inactivated clotting factor (zymogen). When thrombin has been formed in sufficient quantities to overcome the effects of antithrombins and proteinase inhibitors, thrombin cleaves four peptide bonds in fibrinogen, forming soluble monomers and the two negatively charged fibrinogen peptides, A and B. The monomers polymerise linearly and laterally to form an insoluble fibrin network which is stabilised by factor VIIIa.

Figure 4 The activation and inhibition of the coagulation and fibrinolytic system (adapted from Jerling 1998:12)
CHD: Coronary heart disease; F: Factor; a: Activated: Prot: Protein; TF: Tissue factor; Ca: Calsium; PAI-1: Plasminogen activator inhibitor 1; TFPI: Tissue factor pathway inhibitor; FDP: Fibrin degradation products; tPA: tissue plasminogen activator; PF3: Platelet factor 3; APC: Active protein C; PS: Protein S; AT: Antithrombin

Protein C, activated by thrombin, deactivates factor V and factor VIII. Protein S is a co-factor for active protein C ensuring that protein C binds to the platelet membrane surface (Summarised by Jerling, 1998:12-13; Murphy, 1994:336-337; Reilly & Cawley, 1996:11.9-11.10; Vorster *et al.*, 1997; Vorster, 1987:31-49).

4.4 Fibrinolysis

Fibrinolysis is the enzymatic degradation of fibrin clots. The activation and inhibition of the fibrinolytic system is illustrated in Figure 4.

Inactive plasminogen is converted to plasmin by plasminogen activators. Plasmin breaks down the fibrin clot into fibrin degradation products, of which D-dimer is one type. Two types of plasminogen activators are known, namely tPA, stimulated by thrombin, and uPA, synthesised in the kidney. tPA is inactivated by PAI-1 that binds to tPA to form an inactive tPA/PAI-1 complex. Another inactivator of fibrinolysis is α_2-antiplasmin which inactivates plasmin. Active protein C inactivates PAI-1 and therefore induces fibrinolysis (Summarised by Jerling, 1998:13; Murphy, 1994:338-339; Reilly & Cawley, 1996:11.10; Vorster *et al.*, 1997; Vorster, 1987:50-55).

5. THE PATHOGENESIS OF ATHEROSCLEROSIS AND THROMBOSIS: THE ROLE OF LIPIDS, LIPOPROTEINS AND THE HAEMOSTATIC SYSTEM

5.1 Introduction

The pathogenesis of atherosclerosis has been described in several review articles. The discussions of mechanisms differ between the different authors. Moreover, very few authors discuss the role of the coagulation and fibrinolytic system in the atherosclerotic process. The purpose of this section is to provide an overview of the hypothesis of atherosclerosis development which is accepted by most investigators in this field, namely the "response to injury" hypothesis, indicating at the same time the role of the haemostatic system in atherosclerosis development. The atherosclerotic process is illustrated in Figure 5.

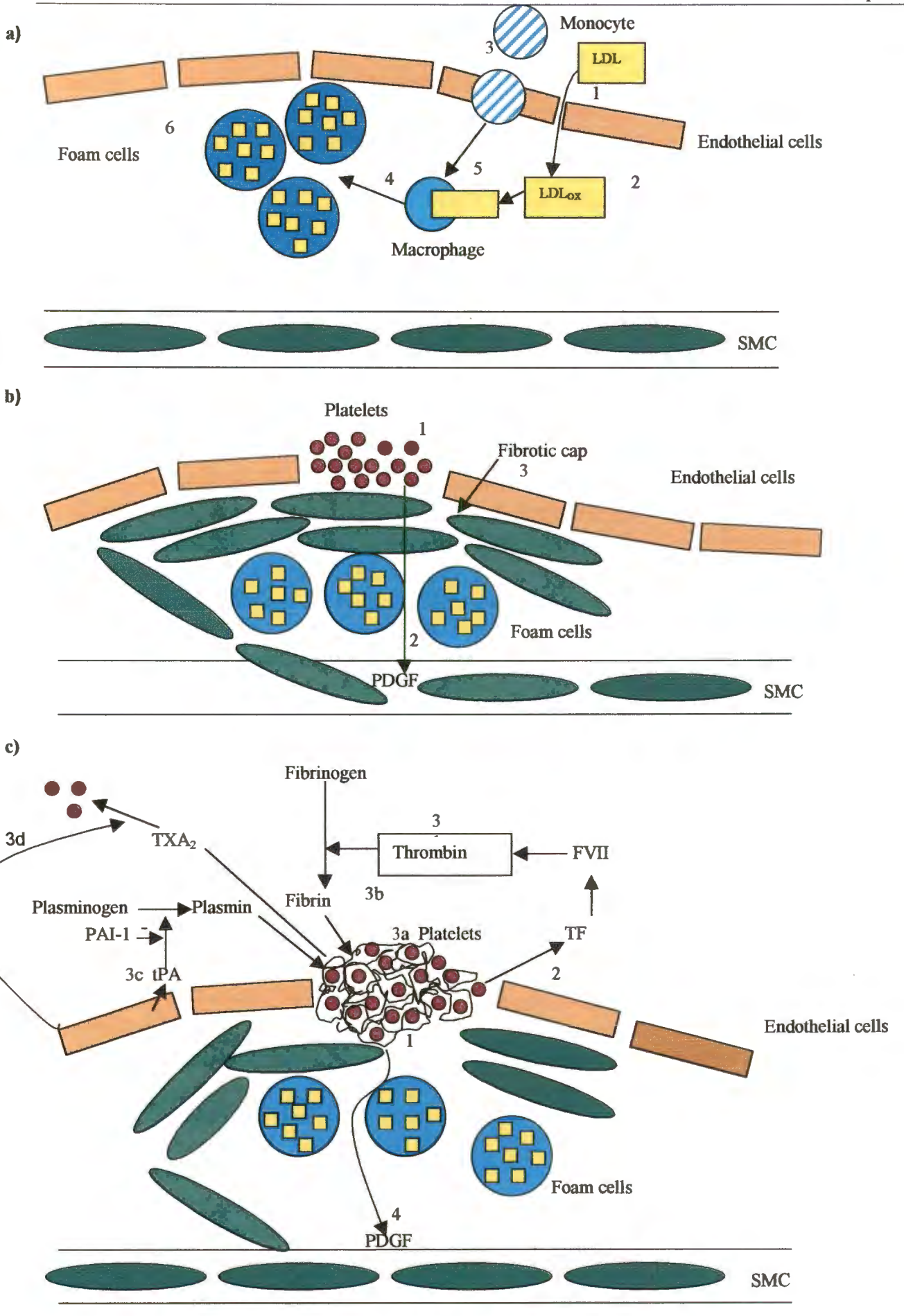

Figure 5 (figure description on page 44)

5.2 The "response to injury" hypothesis

The "response to injury" hypothesis proposes that atherosclerosis is initiated by injury to or stimulation of the endothelium.

In its normal state the endothelium has several physiological functions that retard the development of atherosclerosis. Endothelial cells are joined together to form a semi-permeable barrier that limits the efflux of large molecules such as LDL; it also provides the artery with a non-thrombogenic surface by inhibiting platelet deposition; it releases PGI_2, an endothelium-derived relaxing factor or nitric oxide (EDRF-NO); it secretes factors that inhibit smooth muscle cell migration and proliferation, namely heparin sulphate and EDRF-NO and it provides a surface to which monocytes and lymphocytes cannot adhere. These functions are lost with endothelium injury (summarised by Consigny, 1995).

Some of the many different factors that can damage the endothelium are hypercholesterolaemia, mechanical changes in blood flow, hyperhomocystinaemia, immunological factors, toxins, viruses, diabetes, hypertension, smoking, ischaemia/reperfusion (reviewed by Ross, 1993; Schachter, 1997). Endothelial injury may also be caused by oxidised LDL particles released from macrophages within the subendothelial space. The oxidation of LDL probably occurs within the artery and not in the circulation. The initiating step in the oxidation of LDL is peroxidation of polyunsaturated fatty acids in the LDL lipoprotein. The cells must, however, first generate oxidation potential and one possibility would be to generate free radicals (Steinberg *et al.*, 1989). Free radicals can be generated by normal metabolic processes, high concentrations of metals such as iron and copper, exercise, inflammation, ischaemia/reperfusion, cigarette smoking, environmental pollutants, radiation, ultraviolet light, certain drugs and the ozone (summarised by Langseth, 1995:1; Steinberg *et al.*, 1989).

Atherosclerosis proceeds through a series of pathological stages, namely formation of 1) fatty streaks; 2) fibrous plaques and 3) advanced lesions (summarised by Consigny, 1995).

Fortunately the progression of atherosclerosis can be slowed and even regressed with pharmacological, dietary and exercise intervention (summarised by Schell & Myers, 1997).

Figure 5 The pathogenesis of atherosclerosis (adapted from Consigny, 1995)
a) Fatty streak formation; b) Fibrotic lesion formation; c) Advanced lesion formation.
LDL: Low density lipoprotein; LDL_{ox}: oxidised LDL; SMC: Smooth muscle cells; PDGF: Platelet-derived growth factor; TXA: Thromboxane; PGI: Prostacyclin; tPA: Tissue type plasminogen activator; PAI-1: Plasminogen activator inhibitor-1; FVII: Factor VII; TF: Tissue factor; -: inhibit.

5.3 Fatty streak formation

The earliest visible lesion in the pathogenesis of atherosclerosis is the so-called fatty streak:

1. After injury to the endothelium, the fatty streak begins with the transport of lipoproteins, mainly LDL, into the artery wall, specifically into the subendothelial space by diffusion through the dysfunctional endothelium with increased permeability.

2. LDL becomes oxidised within the arterial subendothelial space by chemical acetylation.

3. LDL_{ox} attracts circulating monocytes. Monocytes adhere in clusters to the endothelium, migrate over its surface and reach the subendothelial space by penetrating at endothelial cell junctions.

4. In the subendothelial space the monocytes are converted to macrophages.

5. The macrophages are scavenger cells that recognise and ingest LDL_{ox} through scavenger receptors. The LDL-rich macrophages then become foam cells which cluster in the subendothelial space to form a fatty streak (reviewed by Consigny, 1995; Hegele, 1996; Ross, 1993; Schachter, 1997).

5.4 Fibrotic lesion formation

1. Increased endothelial disruption occurs because of shear stress placed on the dysfunctional cells from deformation of the arterial wall, or from toxins (free radicals or products of lipid oxidation) released by underlying foam cells.

2. At the sites of cell loss, platelets adhere and release PDGF, which leads to migration of smooth muscle cells (SMC) from the media to the intima and to the proliferation of SMC.

3. At the edge of the fatty streak, the replicating SMC forms a fibrotic cap (reviewed by Consigny, 1995; Hegele, 1996; Ross, 1993; Schachter, 1997).

5.5 Advanced lesion formation

1. The fibrotic lesion may progress into an advanced lesion, in which a thrombus has formed subsequently to fracture or rupture of the fibrotic cap.

2. The coagulation cascade is initiated by exposure of blood to collagen within the plaque and by TF release. TF stimulates the extrinsic pathway through activation of factor VII.

3. A cascade of reactions lead to the formation of thrombin, which has the following actions:

3a. Stimulation of platelet aggregation to form a platelet plug;

3b. catalysation of fibrin formation that stabilises the platelet plug;

3c. stimulation of tPA release that catalyses plasmin formation that breaking down the fibrin clot. PAI-1 can inhibit tPA action; and

3d. stimulation of PGI_2 release that opposes the powerful platelet aggregating and vasoconstrictor properties of TXA_2 that is synthesised.

4. The release of PDGF by platelets lead to further recruitment of SMC.

Depending on the balance between thrombotic and thrombolytic processes, the thrombus may dissolve through initiation of the fibrinolytic system or it may be incorporated into the atherosclerotic lesion, resulting in lesion enlargement that can occlude the artery. It could also be sloughed off and travel as an embolus to a distal vessel (Reviewed by Consigny, 1995; Jerling, 1998:11-13; Murphy, 1994:336-339; Reilly & Cawley, 1996:11.8-11.10; Vorster *et al.*, 1997).

5.6 Concluding remarks

In discussions of the mechanisms involved in the pathogenesis of atherosclerosis, the haemostatic system is often neglected and more attention is paid to the role of lipids and lipoproteins. From the above discussion it is clear that both the lipid and lipoprotein metabolism and the haemostatic system play important roles in the pathogenesis of atherosclerosis.

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CHAPTER 3

ALCOHOL CONSUMPTION AND IT'S RELATIONSHIP WITH RISK FACTORS FOR CORONARY HEART DISEASE IN WHITE SOUTH AFRICANS: THE VIGHOR STUDY

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Abstract

Very little is known about the alcohol consumption of South Africans at risk for coronary heart disease (CHD). This paper therefore describes the mean alcohol consumption and it's association with risk factors for CHD of randomly selected white men (n=1 182) and women (n=1 159), aged 15-64 years, who participated in the VIGHOR study. Alcohol consumption was assessed by a questionnaire, administered by registered dieticians. Systolic and diastolic blood pressures were also measured. Serum lipoproteins and gamma-glutamyltransferase (GGT) concentrations were measured by standard methods. The linear relationship between alcohol intake and the measured variables were estimated by using partial correlations to control for potential confounding factors like age, body mass index, smoking, education and income. The results showed that the reported consumption of alcohol was low if compared to the international literature. Alcohol consumption was significantly positively associated with serum GGT and high density lipoprotein cholesterol levels. A small positive association with systolic and diastolic blood pressure was observed in men. Recommendations for alcohol consumption to decrease CHD risk must thus be done with caution since alcohol consumption can be associated with both risk and protective effects against CHD. Because alcohol forms part of many South African's lifestyle, light-to-moderate drinking together with cessation of smoking and weight control can be recommended for alcohol consumers, except for individuals with alcohol related disorders.

Key words: alcohol, lipoproteins, blood pressure, gamma-glutamyltransferase, VIGHOR

Uittreksel

Baie min is bekend oor alkoholinname van Suid-Afrikaners met 'n verhoogde risiko vir koronêre hartsiektes (KHS). In hierdie artikel word die gemiddelde alkoholinname en verband daarvan met risikofaktore vir KHS in ewekansig geselekteerde mans (n=1 182) en vroue (n=1 159), tussen 15 en 64 jaar oud en wat aan die VIGHOR-studie deelgeneem het, gerapporteer. Alkoholinname is met behulp van 'n vraelys, wat deur geregistreerde dieetkundiges toegepas is, bepaal. Sistoliese en diastoliese bloeddrukke is gemeet. Serum lipoproteïene en gamma-glutamieltransferase (GGT) is met behulp van gestandaardiseerde metodes bepaal. Die liniêre verband tussen alkoholinname en gemete veranderlikes is met behulp van parsiële korrelasies bepaal sodat daar vir ouderdom, liggaamsmassa-indeks, die rookgewoonte, opvoedkundige kwalifikasie en inkomste-vlakke gekontroleer kon word. Die resultate het aangetoon dat die gerapporteerde inname van alkohol, in vergelyking met die internasionale literatuur, laag was. Alkoholinname het betekenisvolle positiewe korrelasies met serum-GGT en hoëdigtheidslipoproteïencholesterol getoon. 'n Klein positiewe korrelasie tussen alkoholinname en bloeddruk is ook in mans waargeneem. Aanbevelings vir inname van alkohol om KHS-risiko te verlaag moet dus met versigtigheid gepaard gaan omdat alkoholinname tot beide KHS-risiko en voordeel kan bydra. Alkoholinname vorm 'n deel van die Suid-Afrikaanse lewenstyl en ligte-tot-matige inname van alkohol gepaardgaande met gewigsverlies en staking van die rookgewoonte kan daarom vir alkohol gebruikers aanbeveel word, maar nie vir persone met alkohol verwante siektes nie.

Introduction

Throughout the world policy makers on governmental and private level are currently beginning to focus on the general alcohol intake or drinking situation in their countries. The major question that faces policy makers is whether drinking in a particular community is heavy enough to be a danger for exacerbating biopsycho-social problems. The proportion of people in South Africa consuming alcohol is gradually increasing (1).

A U-shaped relation between alcohol intake and mortality exists. Consumption of an average of one or two units of alcohol per day is associated with significantly lower all-cause mortality than the consumption of no alcohol, or the consumption of substantial amounts (2, 3, 4). The French have the highest intake of alcohol in the world, and their coronary heart disease (CHD) mortality rates were the second lowest of 21 countries studied (5). Although adverse effects of alcohol intake are well documented, namely certain types of cancer especially breast cancer, hepatic cirrhosis, hypertension, acute death from poisoning, accidents or violence (3, 6, 7), it has a beneficial effect on CHD through effects on serum high density lipoprotein cholesterol (HDL-C) (8, 9, 10, 11, 12, 13, 14, 15), thrombotic factors such as platelet aggregation (16) and plasma fibrinogen (17). It seems however, that the beneficial effects of moderate drinking on cardiovascular mortality may partly (<20%) be neutralised by the deleterious effect of a small increase in blood pressure (18).

In South Africa, very little is known about the alcohol intake of subjects at risk for CHD. This study therefore describes the mean alcohol consumption and it's association with risk factors for CHD in white men and women from the industrialised communities of Witbank and Vanderbijlpark who participated in the Vanderbijlpark Information on Gesondheid/Health, Obesity and Risk factors (VIGHOR) project (19, 20). The data was controlled for potential confounding factors such as age, body mass index (BMI), smoking, education and income.

Subjects and Methods

In 1988 a stratified random sample of 2 885 apparently healthy white men and women between the ages of 10 and 64, from the industrialised communities of Vanderbijlpark and Witbank, participated in part of the VIGHOR project (see references 19-20 for detailed methodology).

For the purpose of this study only data obtained from 1 182 men and 1 159 women, aged 15-64 were used (pregnant and lactating women and children aged 10-14 were excluded from the analyses). The following relevant information for this study was obtained from each subject: A questionnaire, administered by 20 registered and experienced dieticians during personal interviews with subjects, yielded information on educational level (classified as (1) < Std. 6; (2) Std. 6-8; (3) Std. 6-8 plus a trade; (4) Std. 9-10; (5) Std. 9-10 plus a trade; (6) Std 10 plus university training; (7) Std 10 plus any other training); family income per month before taxes (classified as (1) R0; (2) R1-500; (3) R500-1 000; (4) R1 000-1 500; (5) R1 500-2 000; (6) R2 000-2 500; (7) > R2 500; (8) do not know; (9) not applicable); and smoking habits. The physical activity index was determined according to the formula of Sharkey (1979) (21) (See reference 22 for detailed methodology). Participants who indicated that they consumed alcohol were asked about the volume of their habitual daily, weekly, monthly or occasional intake. The daily alcohol consumption was estimated on a basis of an alcohol content (g/v) of 3.1% for beer, 9% for table wine (white/red), 6.5% for light wine, 15.5% for sherry or port and 31.7% for spirits (23). Height and weight measurements from which the BMI was calculated (kg/m^2) were also measured. Two blood pressure measurements were taken by qualified nurses at die left arm, in the sitting position. The nurses were instructed and standardised by the state physician. The first measurement was taken after a rest period of 5 minutes and the second measurement after a few minutes have elapsed. The systolic blood pressure (SBP) was recorded at the first Korotkoff phase and the diastolic blood pressure (DBP) at the fourth Korotkoff phase. A non-fasting venous blood sample for measurements of serum lipids and lipoproteins (see reference 20 for detailed methodology) and gamma-glutamyltransferase (GGT) (24), as guide to alcohol intake, was obtained. The subjects gave written informed consent to participate, and the study was approved by the Ethics Committee of the Potchefstroom University for Christian Higher Education.

Statistical analyses

The SAS® package (25) was used to analyse the data. The means and standard deviations (SD) of men and women according to age groups and different alcohol consumption categories were calculated. The relationship between alcohol intake and the different variables were

estimated by using Pearson correlations. Partial correlations (i.e. R^2-$R1^2$, where R and R1 are the multiple correlations including all variables and when leaving one out) were also calculated with alcohol consumption as dependent variable, to control for age, BMI, educational level, income, physical activity and smoking.

Results

The percentages, mean (SD) of characteristics of subjects according to daily alcohol consumption are summarised in Table 1. More men (66.1%) than women (45%) reported consuming alcohol. Only 0.2% of women in comparison with 6.41% of men consumed more than 28g of alcohol/day (>2 drinks/day), the amount that is recommended by the Committee on Diet and Health, National Research Council, 1989 (26). Mean alcohol consumption among alcohol drinkers was reported to be 13.67(20.5)g/day (1 drink/day) and 4.7(6.2)g/day (3 drinks/week) for men and women respectively. The mean (SD) alcohol consumption according to age groups are shown in Table 2.

Partial correlations between alcohol consumption and blood pressure, serum lipoproteins and GGT were controlled for potential confounding factors such as age, BMI, smoking, physical activity, education and income. The mean (SD) of SBP and DBP of subjects according to daily alcohol consumption categories are summarised in Table 3. Alcohol consumption was positively associated with SBP and DBP in men, but not in women. The association was, however, very small, especially for DBP (R^2 =0.004). Total serum cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels were not significantly associated with alcohol consumption (Table 4). Mean HDL-C concentrations increased with increasing alcohol consumption in men from 0.96mmol/L in abstainers to 1.18mmol/L with intake of more than two drinks/day (23% increase). In women HDL-C concentrations increased from 1.18mmol/L in abstainers to 1.51mmol/L with intake of one drink/day (28% increase). Serum GGT was also positively correlated with alcohol intake. This correlation was, however, very small in the women (R^2=0.004).

TABLE 1: Percentages and mean (SD) characteristics of subjects according to daily alcohol consumption categories

Variable	Alcohol intake (g/day) 0	≤2	>2-4	>4-6	>6-8	>8-10	>10-12	>12-14	>14-28	>28
Drinks/week	0	≤1	1-2	2-3	3-4	4-5	5-6	6-7	7-14	>14
Number:										
M	411	191	90	93	59	67	53	36	126	86
W	662	306	89	42	35	16	16	8	26	3
% of total:										
M	33.9	15.8	7.4	7.7	4.9	5.5	4.4	3.0	10.4	7.1
W	55.0	25.4	7.4	3.5	2.9	1.3	1.3	0.7	2.2	0.2
Age (yr.):										
M	32.6 (14.9)	30.2 (13.0)	33.9 (11.4)	35.6 (12.9)	37.3 (15.1)	36.2 (10.0)	35.5 (12.9)	34.9 (10.8)	37.8 (12.6)	41.3 (13.3)
W	35.9 (14.8)	33.9 (13.0)	40.0 (11.1)	39.0 (11.8)	40.9 (11.5)	39.3 (12.6)	37.3 (10.0)	40.3 (9.7)	49.5 (10.5)	45.67 (9.87)
BMI (kg/m^2):										
M	25.0 (4.58)	24.6 (4.2)	25.6 (3.73)	25.9 (4.03)	25.5 (3.26)	26.4 (4.21)	26.1 (3.77)	26.9 (4.12)	26.1 (3.45)	27.3 (6.24)
W	25.0 (5.18)	24.3 (5.17)	25.0 (4.52)	24.0 (3.63)	24.3 (4.00)	25.7 (5.93)	24.8 (2.46)	25.0 (3.20)	24.2 (3.21)	27.4 (7.78)
Smokers (%):										
M	20.6	29.2	37.8	36.6	44.1	41.8	35.9	55.6	42.1	50.0
W	17.2	19.8	22.5	35.7	31.4	31.3	31.3	43.8	62.5	34.6
Activity @:										
M	24.0 (31.8)	24.4 (29.2)	19.2 (30.0)	20.8 (28.6)	16.1 (26.2)	16.9 (24.9)	20.5 (26.5)	28.0 (29.7)	20.7 (26.0)	15.7 (24.1)
W	15.0 (26.1)	16.2 (24.4)	9.9 (19.2)	13.0 (25.2)	15.2 (22.2)	15.8 (25.1)	25.0 (33.8)	10.3 (17.6)	13.4 (21.3)	10.7 (18.5)
Level of Education#:										
M	3.7 (1.81)	4.21 (1.92)	4.44 (1.69)	5.04 (1.68)	4.9 (1.67)	4.69 (1.66)	5.34 (1.59)	4.39 (1.61)	5.14 (1.68)	4.78 (1.52)
W	3.38 (1.60)	3.89 (1.71)	4.29 (1.75)	4.43 (1.89)	4.11 (1.62)	4.06 (1.98)	4.31 (1.66)	3.63 (1.69)	4.85 (2.03)	5.67 (1.53)
Income#:										
M	5.51 (1.56)	5.63 (1.50)	5.86 (1.45)	5.81 (1.51)	5.47 (1.72)	6.05 (1.42)	5.8 (1.69)	5.97 (1.40)	5.99 (1.44)	6.0 (1.55)
W	3.7 (1.81)	5.69 (1.46)	6.18 (1.45)	5.93 (1.56)	5.86 (1.59)	5.63 (1.63)	6.38 (1.15)	6.38 (0.92)	5.96 (1.28)	6.33 (1.15)

M: men; W: women; BMI: body mass index; #: mean (SD) of classification as described in the methods sections; @: Physical activity index (21)

TABLE 2: Mean (SD) daily alcohol intake (g) according to age groups

Age groups (years)	Men	Women
15-19	(n=201) 1.63(4.83)	(n=183) 0.46(1.12)
20-34	(n=445) 10.29(21.00)	(n=384) 1.80(3.34)
35-44	(n=237) 8.75(13.40)	(n=244) 2.44(6.26)
45-64	(n=299) 11.86(19.84)	(n=348) 2.47(5.20)

TABLE 3: Mean (SD) blood pressure (mmHg) of subjects according to daily alcohol consumption categories

Variable	Alcohol intake (g/day)										$R^{2\#}$	$R^{2\#\#}$
	0	≤2	>2-4	>4-6	>6-8	>8-10	>10-12	>12-14	>14-28	>28		
Systolic												
M	126	125	130	128	127	132	126	129	130	132	0.01*	0.01*
	(15.4)	(14.8)	(14.5)	(14.7)	(11.9)	(15.0)	(13.3)	(15.7)	(14.0)	(16.1)		
W	120	117	116	118	119	117	116	116	126	117	NS	NS
	(16.5)	(14.6)	(15.6)	(12.9)	(18.3)	(16.7)	(15.7)	(14.2)	(19.3)	(11.5)		
Diastolic												
M	79	78	80	81	80	85	81	82	84	84	0.004*	NS
	(11.2)	(11.5)	(10.9)	(11.2)	(11.7)	(10.1)	(8.4)	(10.8)	(9.8)	(13.5)		
W	75.9	74	75	74	76	76	73	82	79	87	NS	NS
	(11.2)	(10.1)	(9.6)	(10.7)	(10.8)	(11.9)	(10.9)	(11.7)	(10.7)	(5.8)		

M: men; W: women; NS: not significant

R^2: partial correlations of variables with alcohol intake (controlled for age, BMI, smoking, physical activity, level of education, income) (#: for the whole group; ##: for the alcohol drinkers; * = p≤0.05)

TABLE 4: Mean (SD) serum lipoprotein and serum GGT levels of subjects according to daily alcohol consumption categories

Variable	Alcohol intake (g/day)										$R^{2\#}$	$R^{2\#\#}$
	0	≤2	>2-4	>4-6	>6-8	>8-10	>10-12	>12-14	>14-28	>28		
TC (mmol/L)												
M	5.33	5.38	5.68	5.55	5.26	6.00	5.72	5.84	5.86	6.04	NS	NS
	(1.35)	(1.27)	(1.37)	(1.39)	(0.98)	(1.23)	(1.48)	(1.15)	(1.25)	(1.29)		
W	5.76	5.62	5.97	5.72	6.09	5.59	6.37	6.03	6.29	6.48	NS	NS
	(1.42)	(1.30)	(1.46)	(0.97)	(2.38)	(1.13)	(2.43)	(1.43)	(1.39)	(0.74)		
HDL-C (mmol/L)												
M	0.96	0.98	0.98	1.01	0.96	1.02	1.02	1.07	1.05	1.18	0.08*	0.07*
	(0.22)	(0.22)	(0.22)	(0.23)	(0.17)	(0.18)	(0.23)	(0.23)	(0.26)	(0.40)		
W	1.18	1.23	1.30	1.37	1.36	1.19	1.40	1.38	1.51	1.40	0.03*	0.02*
	(0.28)	(0.29)	(0.26)	(0.38)	(0.29)	(0.22)	(0.41)	(0.24)	(0.50)	(0.15)		
LDL-C (mmol/L)												
M	3.48	3.60	3.74	3.68	3.32	3.95	3.80	3.80	3.78	3.85	NS	NS
	(1.16)	(1.14)	(1.21)	(1.24)	(0.85)	(1.20)	(1.30)	(0.94)	(1.09)	(1.22)		
W	3.89	3.72	3.94	3.64	4.08	3.50	4.20	3.77	4.03	4.51	NS	NS
	(1.23)	(1.11)	(1.27)	(0.84)	(2.29)	(0.87)	(2.45)	(1.39)	(1.21)	(0.63)		
TG (mmol/L)												
M	2.03	1.78	2.24	1.87	2.11	2.40	2.01	2.48	2.28	2.44	NS	NS
	(1.63)	(1.24)	(1.78)	(1.11)	(0.98)	(1.79)	(1.16)	(2.39)	(1.19)	(2.44)		
W	1.52	1.44	1.60	1.55	1.41	1.95	1.69	1.92	1.64	1.23	NS	NS
	(1.19)	(1.14)	(1.05)	(1.23)	(0.96)	(1.40)	(0.91)	(1.27)	(0.84)	(0.58)		
GGT (mmol/L)												
M	23.0	19.8	24.3	22.2	24.1	31.2	38.2	34.3	34.7	53.4	0.07*	0.07*
	(20.3)	(11.2)	(18.4)	(11.3)	(18.7)	(31.3)	(80.3)	(27.9)	(35.5)	(73.8)		
W	16.7	16.2	20.3	16.5	19.2	17.0	22.1	20.9	28.4	21.7	0.004*	NS
	(13.8)	(11.2)	(27.2)	(9.1)	(9.1)	(5.3)	(19.4)	(13.2)	(30.0)	(11.7)		

M: men; W: women; TC: total cholesterol; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; TG: triglycerides; GGT: gamma-glutamyl transferase; NS: not significant

R^2: partial correlations of variables with alcohol intake (controlled for age, BMI, smoking, physical activity, level of education, income) (#: for the whole group; ##: for alcohol drinkers; * = p≤0.05)

Discussion

This particular industrialised community reported lower levels of alcohol consumption than that reported in the international literature (8, 10, 12). It could be that their intake were indeed lower or that measured intakes were low due to (1) sampling bias and/or (2) difficulty in obtaining accurate information on usual drinking habits because of it's emotional and moral overtones or the fact that respondents may underestimate the amount drunk (2). The mean alcohol intake could also have been underestimated because the questionnaires were administrated by dieticians. In the Caerphilly and Speedwell Collaborative Heart Disease Study (14) the mean alcohol consumption in Caerphilly was 204ml/week with a self-administered questionnaire, while the same questionnaire in Speedwell that was administered by a nurse, yielded a mean alcohol intake of 125ml/week. A raised serum GGT level is a good guide to alcohol intake and a mild elevation is common even with small alcohol consumption (27, 28). The range for normal GGT values is 10-40 IU/L (27). In this study serum GGT increased gradually with increasing alcohol consumption in both men and women. The serum GGT levels remained in the normal range except for men who drank more than two drinks (>28g) per day. This may be an indication that this population did consume low amounts of alcohol. Even if the reported alcohol consumption is underestimated the positive association seen between alcohol consumption and serum GGT concentrations indicates that the measurement of drinking patterns are accurate.

In this study alcohol intake was positively correlated with serum HDL-C levels, independent of age, BMI, physical activity, education or income. This association is well documented in the literature (8, 9, 10, 11, 12, 13, 14, 15) and is due to an increase in both HDL_2 and HDL_3 subfractions (11, 14). Possible meganisms by which alcohol may influence serum HDL-C is through alcohol's ability to induce hepatic microsomal enzyme synthesis. These enzymes are responsible for enhanced synthesis of HDL and thereby increase serum HDL-C concentration. Alcohol may also increase lipoprotein lipase activity, which causes an increased catabolism of very low density lipoprotein cholesterol, thereby raising serum HDL-C (29, 30).

This study showed no association of alcohol consumption with TC, LDL-C or TG concentrations, perhaps because of the low levels of intake. Data on the effects of alcohol on these lipoproteins are scarce. A weak positive relation between alcohol intake and TC has been reported in men (12), but throughout the literature mostly no association is found (14).

An increase in alcohol intake have been reported to increase TG levels and to decrease LDL-C levels (8, 14).

A small but significant positive association between blood pressure (SBP and DBP) and alcohol consumption in men was seen after adjusting for the effects of several confounding factors, namely age, smoking, education, BMI and physical activity (6, 7). A significant association could not be demonstrated for either SBP or DBP in women. Studies have observed either a positive linear, curvilinear, J-shaped or U-shaped association between alcohol intake and blood pressure in both men and women (6, 7, 31, 32, 33). The most desirable risk factor profile appears to be found in those individuals consuming the equivalent of one to two drinks daily (32, 34). In the 1982 Maryland Hypertension Survey, Moore *et al.* (7) observed a J-shaped dose-response association in <50yr olds with abstainers and heavy alcohol consumers having significantly higher blood pressures than moderate alcohol consumers (1-2 beverages per day). In this study only three of the women drank more than two drinks per day. This may explain why a significant association could not be demonstrated. Although our data do not allow distinction between beverage types, Rocha-Silva (1) reported that women in South Africa drink more wine than spirits and beer. Some studies have suggested that the blood pressure elevating effect of wine is lower than the effect of beer plus spirits. Van Leer *et al.* (6) reported a stronger relation between blood pressure and alcohol intake in older than younger men and in male and female smokers than non-smokers. The small association seen in this study might therefore be ascribed to the fact that the effects of smoking and age was controlled for. In one other study a significant association between alcohol consumption and blood pressure for either sex was not found and could not be explained by the authors (35).

Alcohol may be a vasodilator at low doses but a pressor at higher doses (34). Randin *et al.* (36) investigated the potential mechanism of the acute pressor effect of alcohol in normal subjects. They found that the short-term administration of alcohol in a dose equivalent to two or three drinks increased blood pressure by an average of 10mmHg and also increased firing of the sympathetic vasoconstrictor nerves targeted to the skeletal-muscle bed, offsetting any local vasodilative property of ethanol and contributing to the pressor response which seems to be due primarily to an increase in heart rate (34). Other mechanisms that have been suggested include effects on renin, catecholamines, vasoreactivity and/or calcium channels (7).

In this study alcohol consumption had a positive effect on HDL-C levels in both sexes, but a small negative effect on blood pressure levels in men. The balance between risks and benefits of alcohol consumption is a complicated matter. It seems as if light-to-moderate drinking (1-6 drinks per week) is associated with the lowest risk from all-cause mortality, especially from CHD, non-haemorrhagic and ischaemic stroke, and that heavier drinking is associated with an increased risk of death from other causes, namely certain cancers especially breast cancer in women, cirrhosis and haemorrhagic stroke, (3, 4, 37, 38, 39, 40). Moderate drinking may also increase the risk for subarachnoid haemorrhage (37). Thus, it is probably undesirable to encourage the consumption of alcohol for cardioprotective purposes as a public health measure, given the deleterious effects of alcohol, the extent of alcohol abuse and the genetic predisposition to alcoholism in many persons (5). Alcohol, however, forms an important part of many South African's lifestyle. Therefore, responsible, light-to-moderate drinking together with cessation of smoking and weight control can be recommended, except for individuals who should not consume alcohol, such as former abusers, pregnant women, individuals with diseases or other conditions that might be adversely affected by this substance.

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CHAPTER 4

DIETARY COMPOSITION AND BODY MASS INDEX AT DIFFERENT LEVELS OF ADDED SUGAR CONSUMPTION: THE VIGHOR STUDY

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Abstract

Motivation and objective: Little information is available on the effects of added sugar (sucrose) consumption on body mass index (BMI) and nutrient intakes of South Africans that are following typical Western diets, high in fat, low in fibre, and often inadequate in micronutrients. This paper investigates these relationships in a sub-group of randomly selected white men (n=151) and women (n=161), aged 15-64 years, who participated in the VIGHOR project.

Subjects and methods: Dietary intakes were assessed with a 24-hour recall method and converted to nutrients using the MRC's food composition tables. BMI was calculated (kg/m^2). The linear relationships of added sugar intake with BMI and macronutrients were estimated by using partial correlations, controlling for potential confounding factors such as age, physical activity, smoking, alcohol consumption, energy intake and BMI (for macronutrients).

Results: The results showed that added sugar intake was not associated with BMI, and negatively associated with fat and protein intake. In men, the associations between added sugar intake and most of the micronutrients took on inverse U-shaped curves. Except for vitamin D, however, the micronutrient intakes were still >67% of the recommended dietary allowances. Women, probably because of their low energy intakes, were more prone to nutrient inadequacies with increased sugar intake, especially with regard to fibre, iron, magnesium, potassium, copper, thiamine and vitamin B_6.

Recommendation: It is suggested that the message regarding sugar consumption to this population and others moving towards "westernised" diets should be that added sugars can be consumed in moderation. Women with low energy intakes should be cautious not to displace nutrient- and fibre-rich foods with sugary foods.

Key words: Added sugar intake, sucrose intake, dietary sugar intake, body mass index (BMI), nutrient intake

Introduction

In order to formulate practical, positive, affordable and therefore sustainable guidelines regarding sugar (sucrose) consumption, it is important to know what the effects of added sugar consumption are in different disease conditions and also on the nutrient content of current South African diets. Large segments of our population are at present in a process of a "nutrition transition" that involves a change to a more palatable "westernised" diet which contains more animal products, fat, and refined carbohydrate foods such as sugar and less dietary fibre. This diet is currently being eaten by urban populations in South Africa.[1]

Sugar is often implicated as the villain in weight gain. Walker[2] reviewed the literature in 1975 and concluded that sugar intake does not correlate with proneness to obesity. Many epidemiological studies after 1975 could not demonstrate a direct association between high consumption of sugar and body weight[3,4] and some even reported an inverse relationship.[5,6] Yet advice to decrease body weight still includes avoidance or restriction of sugar intake (S M Hanekom, W Oosthuizen – unpublished report to the SA Sugar Association, 1997).

It is also important to consider the role of added dietary sugar on macro- and micronutrient intakes. The inverse association between added sugar and fat consumption is well documented.[3,5,7,8] Only a few studies have examined the effect of added sugar consumption on micronutrient and fibre intake. The available data show no consistent patterns.[5, 7,9-11] Clearly, more research is needed to clarify the role of sugar in adequacy of micronutrient intakes.

The objectives of this study were to investigate the relationship between added sugar consumption and body mass index (BMI) and the effect of added sugar consumption on the macro- and micronutrient intake patterns in subjects following typical Western diets with borderline micronutrient inadequacies. The target population was white men and women from the industrialised communities of Witbank and Vanderbijlpark who participated in the Vanderbijlpark Information on Gesondheid/Health, Obesity and Risk factors (VIGHOR) project. The VIGHOR participants followed a typical Western diet, high in fat, low in fibre with inadequate micronutrient intakes.[1]

Subjects and Methods

In 1988 a stratified random sample of 2 885 apparently healthy white men and women between the ages of 10 and 64 years, from the industrialised communities of Vanderbijlpark and Witbank voluntarily participated in the VIGHOR project (see Van der Merwe *et al.*[12] and Vermaak *et al.*[13] for detailed methodology). Every 7th subject seen, aged 15-64 years (151 men and 161 women), participated in a dietary survey. The 24-hour recall method was administered by experienced dieticians to assess daily dietary intakes and included all days of the week (see Vorster *et al.*[1] for detailed methodology). Nutrient intakes were analysed by using the Medical Research Council (MRC)'s mainframe computer programme, based on the MRC Food Composition Tables.[14] Height and weight measurements from which the BMI was calculated (kg/m^2) were measured with standard methods.[12] Added sugar refers to the sugar added to food, and does not include sugars naturally present in the food, for example lactose in milk and fructose in fruit.[14] Information about physical activity, smoking and alcohol consumption (see Oosthuizen *et al.*[15] for detailed methodology) was also used for this investigation, because of their role as potential confounding factors.[4,16] The subjects gave written informed consent to participate, and the study was approved by the Ethics Committee of the Potchefstroom University for Christian Higher Education.

Statistical analyses

The SAS® package[17] was used to analyse the data. The means and standard deviations (SD) of BMI and nutrient intake, stratified for sex, age (15-24 and 25-64 years) and tertiles of added sugar intake as a percentage of total energy intake, were calculated. The relationship between sugar intake and BMI as well as macronutrient intakes were calculated using partial correlations (i.e. R^2-$R1^2$, where R and R1 are the multiple correlations including all variables and when leaving one out). Added sugar intake as percentage of total energy intake was the dependent variable while age, physical activity, smoking and alcohol consumption were controlled for. In addition, BMI was controlled for when the relationships between sugar intake and macronutrient intakes were estimated. The associations between added sugar intake and some of the micronutrients took on inverse U-shaped curves. Correlation coefficients could therefore not be calculated for these variables.

Results

The change in BMI with increasing consumption of added sugar as percentage of total energy is illustrated in Fig. 1. The mean (SD) BMI of 15-24-year-old men was 23.26 (3.63), that for 25-64-year-old men 26.92 (3.92), that for 15-24-year-old women 21.64 (3.45), and that for 25-64-year-old women 25.41 (5.05). Partial correlation (controlled for age, physical activity, smoking, alcohol consumption and total energy intake), could not demonstrate any significant correlations between sugar intake and BMI.

The mean (SD) daily energy intake and energy distribution among the different macronutrients by tertiles of added sugar intake as percentage of total energy are summarised in Table I. Mean daily energy intakes tended to increase across tertiles of added sugar intake, but remained below the recommended levels of 12 MJ and 9.2 MJ for adult men and women, respectively.[18,19] Added sugar intake was negatively associated with total fat and saturated fat intake in men and protein and fibre intake in women. Weak negative associations were shown for mono-unsaturated fat (MUFA), polyunsaturated fat (PUFA) and cholesterol.

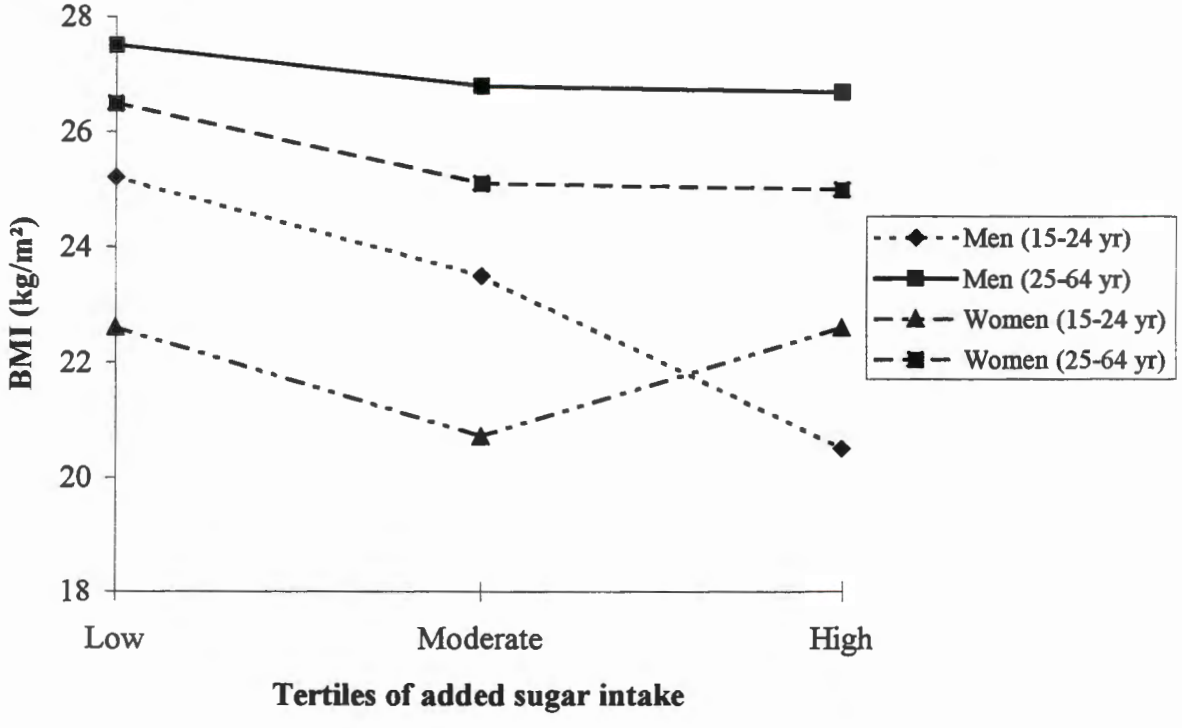

Fig. 1. Mean body mass index by tertiles of added sugar intake as a percentage of total energy.

Table I **Mean (SD) daily energy distribution in the diets by tertiles of added sugar intake as a percentage of total energy and partial correlation coefficients (R^2).**

Nutrient	Men							Women						
	15-24 (years)			25-64 (years)			R^2	15-24 (years)			25-64 (years)			R^2
	T1	T2	T3	T1	T2	T3		T1	T2	T3	T1	T2	T3	
N	9	18	9	28	59	28	-	10	20	10	30	61	30	-
Energy (MJ)	9.3 (4.9)	12.1 (3.3)	12.3 (3.1)	9.6 (4.2)	10.7 (3.8)	11.2 (3.3)	-	8.0 (3.2)	8.2 (2.4)	9.4 (5.3)	5.7 (2.6)	6.3 (2.2)	6.2 (2.0)	-
Added sugar (%)	6.3 (4.3)	16.4 (2.2)	26.1 (6.5)	5.6 (3.4)	15.2 (3.3)	29.1 (9.4)	-	7.1 (2.8)	17.0 (3.4)	30.4 (5.9)	3.4 (2.4)	13.3 (4.0)	27.8 (6.5)	-
Total fat (%)	44.1 (8.3)	35.7 (6.8)	32.2 (4.3)	38.3 (8.1)	39.3 (7.6)	29.1 (8.6)	0.23	41.3 (7.7)	39.3 (7.4)	34.5 (6.7)	34.2 (12.3)	38.1 (7.9)	30.9 (7.1)	0.06
SFA (%)	18.2 (6.8)	12.3 (4.1)	12.7 (2.4)	14.8 (3.8)	14.6 (4.0)	11.1 (3.9)	0.17	16.4 (4.6)	14.2 (3.6)	12.9 (4.1)	11.7 (5.1)	13.6 (3.8)	12.0 (3.7)	0.01
MUFA (%)	14.8 (3.8)	12.3 (3.5)	11.0 (2.8)	13.9 (4.1)	14.7 (3.6)	10.5 (3.8)	0.12	14.7 (3.9)	13.7 (3.2)	12.4 (3.1)	11.6 (5.9)	13.0 (3.9)	11.1 (3.5)	0.03
PUFA (%)	7.8 (4.4)	6.9 (2.7)	5.4 (1.7)	6.4 (3.2)	6.6 (2.6)	4.9 (2.1)	0.05	6.6 (3.0)	7.9 (3.9)	6.4 (2.8)	7.4 (3.8)	8.3 (4.6)	5.0 (2.4)	0.03
Total protein (%)	18.0 (4.2)	14.3 (3.1)	11.7 (2.0)	16.8 (3.3)	15.6 (3.8)	13.5 (2.9)	0.14	17.1 (3.6)	14.8 (3.2)	10.8 (2.7)	19.1 (5.1)	15.9 (3.8)	12.9 (4.5)	0.27
Animal protein(%)	14.4 (5.1)	9.4 (3.5)	7.6 (2.1)	12.7 (3.9)	11.7 (4.2)	9.8 (3.2)	0.09	12.9 (4.0)	10.8 (3.5)	7.9 (3.1)	13.6 (5.4)	11.9 (4.3)	9.3 (5.1)	0.13
Total CHO (%)	41.1 (9.5)	52.8 (7.5)	59.8 (5.6)	40.4 (11)	45.3 (7.0)	58.1 (9.2)	0.43	44.4 (8.5)	48.8 (7.4)	57.5 (6.9)	51.1 (13.9)	48.4 (8.8)	58.0 (10)	0.14
Fibre g/4.18 MJ	7.0 (3.8)	9.3 (4.4)	6.0 (2.8)	6.9 (3.0)	6.8 (3.4)	5.7 (2.3)	0.02	8.1 (3.2)	7.0 (2.8)	4.7 (3.8)	12.8 (7.0)	8.1 (4.0)	6.0 (3.7)	0.17
Cholesterol g/4.18 MJ	206 (62)	140 (80)	104 (35)	189 (120)	160 (99)	110 (51)	0.07	206 (202)	159 (97)	114 (60.6)	204 (120)	168 (124)	118 (99)	0.05

T: tertile; T1: low percentage sugar intake of total energy; T2: moderate percentage sugar intake of total energy; T3: high percentage sugar intake of total energy; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; CHO: carbohydrate.

R^2: partial correlation of variables with sugar intake (controlled for age, physical activity, smoking, alcohol consumption, energy intake and body mass index).

The mean (SD) daily micronutrient intakes by tertiles of added sugar intake as percentage of total energy are summarised in Table II. In men, most of the micronutrients tended to stay above 100% of the recommended dietary allowances (RDA) (or reference nutrient intakes (RNI) in the case of sodium and copper), irrespective of added sugar intake, e.g. iron, phosphorus, sodium, copper, riboflavin (15-24 years), nicotinic acid (25-64 years), folate, vitamin B_{12} (25-64 years), ascorbic acid and vitamin E. Magnesium, potassium and thiamine intakes tended to be <100% of the RDA (or RNI in the case of potassium) across tertiles of added sugar intake. The associations between added sugar intake and some of the

Table II **Mean (SD) daily micronutrient intakes by tertiles of added sugar intake as a percentage of total energy**

Nutrient	Men						Women					
	15-24 (years)			25-64 (years)			15-24 (years)			25-64 (years)		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Calcium (mg)	1253 (1285)	973 (546)	930 (485)	682 (377)	852 (566)	607 (320)	953 (376)	624 (417)	602 (400)	548 (243)	582 (295)	508 (364)
Iron (mg)	9.9 (4.3)	16.9 (9.6)	16.4 (11.0)	14.1 (8.5)	12.7 (6.9)	13.2 (5.7)	10.2 (4.9)	9.6 (3.4)	8.8 (4.5)	9.9 (4.7)	7.8 (3.4)	7.6 (5.0)
Magnesium (mg)	334 (188)	349 (129)	314 (117)	306 (132)	325 (144)	308 (132)	242 (78)	238 (78)	237 (131)	261 (144)	224 (89)	188 (83)
Phosphorus (mg)	1614 (1158)	1595 (523)	1297 (480)	1338 (591)	1453 (647)	1200 (459)	1302 (584)	1079 (390)	932 (542)	951 (318)	923 (338)	752 (326)
Potassium (mg)#	3056 (1983)	3207 (916)	3134 (944)	3112 (1602)	3288 (1278)	3183 (1322)	2417 (951)	2335 (708)	2342 (1261)	2538 (1162)	2338 (892)	1897 (677)
Sodium (mg)#	2440 (1322)	2909 (1113)	2904 (1223)	2025 (1050)	2500 (1428)	1881 (974)	1854 (722)	1910 (930)	1624 (953)	1564 (986)	1543 (813)	1308 (721)
Zinc (mg)	13.6 (7.6)	15.3 (6.3)	11.4 (4.4)	15.7 (11.1)	14.1 (8.9)	13.5 (5.7)	11.9 (9.7)	9.5 (3.9)	8.3 (5.3)	8.5 (3.9)	8.8 (4.8)	7.1 (4.1)
Copper (mg)#	1.2 (0.7)	1.6 (0.6)	1.7 (0.4)	1.3 (0.6)	1.5 (1.1)	1.6 (0.9)	1.0 (0.3)	1.2 (0.4)	1.2 (0.6)	1.5 (1.6)	1.0 (0.4)	0.8 (0.4)
Vitamin A (RE)	750 (396	1427 (980)	1211 (1007)	1085 (1483)	1383 (1945)	984 (1100)	791 (442)	839 (784)	582 (654)	1180 (1725)	973 (1219)	596 (597)
Thiamine (mg)	1.2 (0.7)	1.5 (0.8)	1.4 (0.9)	1.2 (0.7)	1.4 (1.1)	1.1 (0.6)	1.0 (0.4)	1.0 (0.4)	0.8 (0.4)	1.1 (0.6)	0.8 (0.5)	0.6 (0.4)
Riboflavin (mg)	2.2 (2.1)	2.3 (1.6)	2.2 (1.4)	1.8 (1.1)	2.0 (1.5)	1.4 (0.7)	1.7 (0.8)	1.4 (0.5)	2.0 (1.9)	1.5 (1.0)	1.3 (0.5)	1.1 (0.8)
Nicotinic acid (mg)	17.2 (9.1)	23.7 (11.3)	22.8 (12.6)	22.2 (12.5)	23.3 (13.6)	20.1 (8.2)	13.4 (4.9)	15.9 (6.6)	12.5 (7.4)	15.8 (6.6)	12.6 (5.6)	10.8 (6.1)
Vitamin B_6 (mg)	1.3 (0.8)	2.0 (1.2)	1.8 (0.9)	2.0 (1.1)	1.8 (1.4)	1.5 (0.7)	1.3 (0.6)	1.3 (0.6)	1.4 (0.9)	1.4 (0.7)	1.1 (0.5)	0.9 (0.6)
Folate (µg)	237 (155)	282 (188)	203 (100)	268 (231)	228 (164)	201 (115)	172 (85)	177 (83)	169 (112)	202 (121)	177 (88)	128 (85)
Vitamin B_{12} (µg)	7.1 (4.9)	6.3 (2.8)	1.9 (2.3)	7.5 (5.9)	7.6 (7.9)	5.8 (3.1)	5.6 (4.1)	5.0 (2.3)	3.8 (3.3)	6.6 (14.9)	4.7 (3.5)	3.7 (3.5)
Ascorbic acid (mg)	75.2 (99.8)	96.9 (145)	131 (138)	123 (179)	86 (120)	67.5 (85.9)	43.0 (24.8)	64.7 (62.7)	100 (120)	72.7 (64.9)	41.2 (40.8)	60.4 (98.1)
Vitamin D (µg)	5.3 (4.6)	4.8 (3.3)	3.9 (4.3)	4.0 (4.2)	4.8 (5.1)	2.7 (2.3)	6.9 (14.3)	3.5 (2.2)	4.2 (3.8)	4.1 (3.2)	3.8 (3.7)	2.3 (2.9)
Vitamin E (mg)	21.5 (17.3)	22.0 (11.8)	19.5 (13.2)	17.4 (12.5)	19.6 (17.7)	16.9 (10.0)	13.3 (8.9)	15.7 (11.4)	12.7 (9.3)	13.8 (12.2)	15.3 (8.5)	9.9 (7.1)

#: United Kingdom's reference nutrient intakes (RNI)[20]

< 100% of the recommended dietary allowances (RDA)[18]

<67% of the RDA

T: tertile; T1: low percentage sugar intake of total energy; T2: moderate percentage sugar intake of total energy; T3: high percentage sugar intake of total energy.

micronutrients tended to take on inverse U-shaped curves with lower nutrient intakes in tertiles 1 and 3 of sugar intake, e.g. calcium (25-64 years), magnesium, phosphorus (25-64 years), potassium, zinc (15-24 years), vitamin A (26-64 years) and thiamine. Negative linear trends to <100% of the RDA were seen for vitamin B_{12} in 15-24-year-old men and for zinc, riboflavin and vitamin B_6 in 25-64-year-old men. Vitamin D intakes tended to decrease to levels <67% of the RDA with increased sugar intake. In young men, vitamin A and nicotinic acid intakes tended to increase from <100% of the RDA to >100% of the RDA with increased sugar intake.

In women, negative linear trends between added sugar and micronutrient intakes could be seen. Some of the micronutrients decreased from levels >100% of the RDA to <100% of the RDA, e.g. phosphorus and vitamin A, thiamine and zinc in 15-24-year-old women and copper, riboflavin, nicotinic acid and folate in 25-64-year-old women. Increased sugar intake tended to cause a further decrease in some of the already inadequate (<100% of the RDA) micronutrient intakes to <67% of the RDA, e.g. calcium, potassium, vitamin D and magnesium, as well as zinc, copper, thiamine and vitamin B_6 in 25-64-year-old women. Vitamin B_6 (15-24 years) and sodium (25-64 years) tended to be <100% and iron <67% of the RDA, irrespective of sugar intake. Only vitamin B_{12} and vitamin E as well as sodium, riboflavin and folate in 15-24-year-old women remained above 100% of the RDA across tertiles of added sugar intake.

Discussion

Nutrient intake data obtained with the 24-hour recall method do not describe an individual's usual intake. The 24-hour recall method can, however, be used to assess the mean daily intakes of groups consisting of at least 50 respondents.[21] The 25-64-year-old groups consisted of adequate numbers of respondents (115 men and 121 women). The 15-24-year-old groups numbered less than 50 (36 men and 40 women), but because the same trends reported in the literature were demonstrated in these groups the results are shown. The results for these two groups should, however be interpreted with caution.

Underreporting of nutrient intakes, especially by overweight and obese subjects, is usually of great concern when the relationship between BMI and nutrient intakes is examined. Vorster *et al.*[1] stated that the mean reported intakes obtained from the VIGHOR participants were probably true reflections of intakes. Nutrient intakes of subjects who claimed that the 24-hour recalls were not representative of their usual diets were compared with subjects whose 24-hour recalls were representative. No statistically significant differences (data not shown) could be demonstrated and the data of the two groups were therefore combined.

As reported for other international communities[3,4] an association between added sugar intake and BMI could not be demonstrated in this industrialised community. Some studies even reported an inverse relationship.[5,6,22] Four possible mechanisms may explain this lack of association. Firstly, Flatt[23] stated that weight stability occurs when the mixture of fuel oxidised is equal to the mixture of the nutrients consumed. Carbohydrate intake promotes carbohydrate oxidation and energy expenditure, while fat intake produces minimal increase in fat oxidation and energy expenditure, resulting in positive fat balance.[23-25] Less metabolic energy is also needed to store dietary fat, with an energy cost of only 3% of energy intake, whereas the cost of storing dietary carbohydrate as body fat requires the expenditure of 23% of ingested energy.[26] Dietary fat is therefore stored in the adipose tissue much more efficiently than carbohydrate. Secondly, carbohydrate has an increased satiating power relative to fat, especially in obese subjects.[27,28] Thirdly, it is possible that the overweight subjects might have cut down on sugar intake because of the common belief that sugar consumption may be responsible for weight gain. Fourthly, the possibility can not be excluded that because the 24-hour recall method is not representative of usual intakes, overweight subjects with habitually high sugar intakes may have had moderate or low intakes on the day in question.

It is important to note that in this, as well as other epidemiological studies[3-6,22] mean energy intakes were within the recommended levels of 12 MJ and 9.2 MJ for adult men and women, respectively.[19] One cannot ignore the possibility that added sugar in excess may still contribute to overweight. Data from Acheson *et al.*[26] suggest that if massive amounts of carbohydrate continue to be ingested glycogen stores becomes saturated (~500g) and *de novo* lipogenesis begins in order to dispose of excess carbohydrate. Flatt[23] hypothesised that because total substrate oxidation in the body is determined by energy need and the rates of amino acid and carbohydrate oxidation are determined primarily by protein and carbohydrate intakes, the rate of fat oxidation must be set largely by the gap between total energy expenditure and the energy

intake in the form of carbohydrate and protein. If carbohydrate is consumed in excess it may lead to positive fat balance through its sparing effect on fat oxidation.[29]

In this study daily added sugar intake was negatively associated with fat intake, especially in men. This association is well documented.[3,5,7,8,10,30] A decrease in sugar consumption can therefore be expected to lead to an increase in fat intake. Health advice to reduce fat and sugar intake simultaneously may therefore be extremely difficult to achieve. Higher intakes of added sugar were inversely associated with protein intakes, especially in women. This inverse association was also reported by Baghurst *et al.*[7]

It is important to consider the possibility that high consumption of added sugar may displace micronutrient-rich foods and other important macronutrients, such as fibre, in the diet. Very few studies have examined this possible nutrient-diluting effect of increased sugar consumption, and the available data show no consistent patterns.

The inverse association between added sugar and dietary fibre intakes in women in this study was also observed in two other studies.[5,7] Fibre intakes by both men and women in this study, however, did not meet the prudent dietary guidelines of >25-30g/day,[31,32] irrespective of sugar intake.

Before discussing the effects of sugar intake on micronutrient intakes, it is important to realise that the greatest part of the South African food composition tables currently in use[15] are compiled from different sources and may not always be a good reflection of South African foods. Data on dietary composition, especially micronutrients, should therefore be interpreted with extreme caution.

Sugar intake did not constitute a major risk to nutritional inadequacy in men, especially young men. When using <67% of the RDA as standard, micronutrient intakes were adequate, irrespective of sugar intake. Nutrients that tended to decrease linearly to <100% of the RDA with increased sugar intake were zinc (25-64 years) and vitamin B_{12} (15-24 years). Vitamin D intake levels decreased to <67% of the RDA with increased sugar intake. Many of the micronutrient intakes were <100% of the RDA (or RNI in the case of potassium) with both low and high consumption of sugar, e.g. magnesium, potassium, thiamine, vitamin B_6 and calcium (25-64 years). The low micronutrient intakes at low sugar consumption levels may well be explained by the higher fat intake of these subjects. Lower intakes of most vitamins and

minerals with high-fat diets were reported by Baghurst *et al.*[33] The subjects who followed a high-fat diet probably consumed inadequate amounts of vegetables and fruit. Gibney *et al.*[8] reported that high fat consumers had lower intakes of fruit. Lewis *et al.*[3] reported lower intakes of 11 vitamins and minerals by high consumers of added sugar compared to moderate consumers, but most of these nutrients remained above 100% of the RDA. The COMA-report,[9] could not demonstrate significant trends in micronutrient intake among men, while Baghurst *et al.*[7] reported inverse linear trends for vitamin B_6, folate, magnesium and weaker trends for vitamin B_{12}, sodium, calcium and zinc.

Women were more prone to nutrient inadequacy with increased sugar intake, especially with regard to intake of calcium, iron, magnesium, potassium, zinc, copper, thiamine, vitamin B_6 and vitamin D. Mean iron intakes were <67% of the RDA, irrespective of added sugar consumption. The COMA report[9] demonstrated the same trend for iron, but no significant trends were seen by Baghurst *et al.*[7] Baghurst *et al.*[7] showed strong inverse linear trends for vitamin B_6, folate, magnesium, nicotinic acid and weaker, but still significant trends, for vitamin A, vitamin C, vitamin B_{12}, potassium and zinc. He reported that the nutrients that were most as risk of decreasing to levels <70% of the UK's recommended dietary intake levels, were zinc, folate, magnesium and calcium.

In our study micronutrient intakes were highest in men with mean added sugar intakes of 16.4% (117g/day) (15-24 years) and 15.2% (96 g/day) (25-64 years) of total energy intake, while in women most of the micronutrient intakes were highest with the lowest or moderate intakes of added sugar, corresponding to mean added sugar intakes of 7.1-17% (33-82g/day) (15-24 years) and 3.4-13.3% (12-50g/day) (25-64 years) of total energy intake. The literature suggests that micronutrient intakes are highest or do not vary with intake of added sugar over the ranges of approximately 4-12% for women and 6-16% for men.[5,7,9] Bolton-Smith[34] stated that for general populations (men and women combined) with an adequate energy intake, nutrient adequacy can be achieved across a wide range, 4-16% energy, from dietary sugar.

In conclusion, daily added sugar intake was not associated with increased BMI in this industrialised community. In men, it did not constitute a major risk for nutrient inadequacy. High added sugar intake did, however, contribute to the already nutrient inadequate diets of the women. Women were more at risk for nutrient inadequacy, because of their low energy intakes.

Advice to avoid or restrict sugar consumption will probably not correct an already nutrient inadequate diet, and in men low consumption may even have the effect of increasing fat intake and lowering micronutrient intake. Women with low energy intakes should be careful not to displace nutrient- and fibre-rich foods with sugary foods.

The message regarding sugar consumption for this population and others that are moving towards "westernised" diets should be a positive, practical one that sugar can be consumed in moderation (4-16% of total energy intake) within diets that comply to recommended energy and micronutrient intakes.

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CHAPTER 5

LECITHIN HAS NO EFFECT ON SERUM LIPOPROTEIN, PLASMA FIBRINOGEN AND MACRO MOLECULAR PROTEIN COMPLEX LEVELS IN HYPERLIPIDAEMIC MEN IN A DOUBLE-BLIND CONTROLLED STUDY

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Abstract

Objective: To examine the effects of lecithin on serum lipoprotein, plasma fibrinogen and macro molecular protein complex (MPC) levels.

Subjects and study design: Twenty free living hyperlipidaemic men participated in this double-blind study which controlled for possible indirect effects. The subjects were randomly assigned to one of three treatments: frozen yoghurt ór frozen yoghurt with 20g soya bean lecithin ór frozen yoghurt with 17g sunflower oil. Sunflower oil was used to control for the increased energy and linoleic acid intake from lecithin. Yoghurt served as the "vehicle" for the lecithin and sunflower oil and yoghurt alone was given to one group to control for possible effects due to the yoghurt "vehicle", as well as other environmental influences. Variables were measured with standard methods twice at baseline and after 2 and 4 weeks of treatment.

Results: Plasma linoleic acid levels increased significantly with lecithin and sunflower oil treatments indicating that compliance to the treatments were obtained. Lecithin treatment did not have significant effects on serum total cholesterol (TC), triglyceride, high density lipoprotein cholesterol, low density lipoprotein cholesterol, apoprotein A, apoprotein B or lipoprotein (a) levels. Plasma fibrinogen and MPC levels were also not affected by lecithin therapy. Sunflower oil treatment resulted in significant increased body weight, serum TC and decreased MPC levels.

Conclusion: Lecithin treatment had no independent effects on serum lipoprotein, plasma fibrinogen or MPC levels in hyperlipidaemic men.

Sponsorship: Dry bean Producers Organisation (South Africa); Chempure cc (South Africa); SACCA, Pty. Ltd. (South Africa).

Descriptors: lecithin, phospholipids, lipoproteins, lipids, fibrinogen, macro molecular protein complex

Introduction

Lecithin (phosphatidylcholine) is choline containing phospholipids which also includes glycerol esterified with fatty acids (Gurr, 1993:79). Lecithin has emulsifying properties and is an essential component of biomembranes and lipoproteins. Liver, egg yolk and soya beans are especially rich sources of lecithin (Mahan & Arlin, 1992:49). Most of the commercial lecithin brands claim hypocholesterolaemic effects and are therefore prescribed by health practitioners for treatment of increased lipoprotein levels. The assumption that lecithin lowers lipoprotein levels is based on evidence from studies done from the 1940s to the early 1980s (Davies & Murdoch, 1959; Morrison, 1958; Saba *et al*, 1978; Steiner & Domanski, 1944; Tompkins & Parkin, 1980) and most of these studies were poorly designed with controversial results. Some of the major design errors were the following: Firstly, small, heterogenic study groups were used, for example a study group of 8 consisting of men and women with different types of dyslipidaemia including familial hypercholesterolaemia, hypercholesterolaemia, combined hyperlipidaemia and normal lipidaemia (Childs *et al*, 1981; Davies & Murdoch, 1959; Greten *et al*, 1980; Saba *et al*, 1978; Tompkins & Parkin, 1980). Secondly, most studies lacked an appropriate control group (Cobb *et al*, 1980; Davies & Murdoch, 1959; Saba *et al*, 1978, Ter Welle *et al*, 1974; Tompkins & Parkin, 1980). Knuiman *et al* (1989) highlighted four out of twenty four studies that attempted to control for the fatty acid component of lecithin namely, the studies done by Greten *et al* (1980), Childs *et al* (1981), Prack *et al* (1983) and Kesaniemi & Grundy (1986). These studies could, however, not demonstrate any independent effects of lecithin on serum cholesterol. Thirdly, in the studies where control groups were included with cross-over designs, no or too short washout periods existed between control (soya bean oil or safflower oil) and lecithin treatments (Greten *et al*, 1980; Kesaniemi & Grundy, 1986). Fourthly, control and lecithin treatment periods were not randomised with no reference group either. For example, all the subjects will follow the control diet first and then switch to the experimental diet or *vice versa* (Childs *et al*, 1981; Greten *et al*, 1980; Kesaniemi & Grundy, 1986; Ter Welle *et al*, 1974).

Plasma fibrinogen is an independent risk factor for coronary heart disease (CHD) and is positively associated with total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), triglycerides (TG) and negatively associated with high density lipoprotein cholesterol (HDL-C) (Ernst & Resch, 1993). A macro molecular protein complex (MPC) which form complexes with the fibrin network in blood clots, increasing clot mass en decreasing clot fibrinolysis was

described. It is hypothesized that high MPC levels increase thrombotic risk (Lipinski *et al,* 1995). Veldman (1996) showed that MPC levels can be manipulated through dietary intervention in a study where pectin, a soluble dietary fibre component, decreased MPC levels significantly. No information on the effects of lecithin on plasma fibrinogen or MPC levels could be found.

In this study the effect of a new, pure (96% phospholipids) soya bean lecithin product on serum lipoproteins, plasma fibrinogen and MPC levels were investigated by using a double-blind study design in which possible indirect effects were controlled for.

Methods

Subjects

The study was approved by the Ethics Committee of the Potchefstroom University for Christian Higher Education and an informed consent form was signed by each participant. Twenty-one hyperlipidaemic men with a mean (±standard deviation) age of 48.25±9.75 y who regularly attended the Lipid Clinic at the Potchefstroom University, participated in this study. The study was done under free living conditions. The exclusion criteria were: smoking, baseline serum TC levels <5.2mmol/L, baseline serum TG levels >5mmol/L, familial hypercholesterolaemia, baseline fasting blood glucose >6.7mmol/L, body mass index (BMI) >30kg/m^2, and use of lipid lowering drugs.

Study design

At entering the Lipid Clinic all subjects were prescribed a high-fibre, low-fat diet. Compliance to the diet varied. However, all but two subjects were stabilised on their individual adapted diets. Subjects were paired off into three groups of seven subjects each according to their baseline serum TC levels, age and BMI. The groups were then randomly assigned to one of three treatments, namely: 1) 175g/d of frozen yoghurt; 2) 175g/d of frozen yoghurt with 20g soya bean lecithin and 3) 175g/d of frozen yoghurt with 17g sunflower oil. The compositions of the three treatments are summarised in Table 1. By mixing the sunflower oil and soya bean lecithin into the yoghurt, blindness was ensured. Low fat yoghurt was chosen as the "vehicle" for the lecithin and sunflower oil because it is compatible with the low-fat diet. Yoghurt alone

was given to one group to control for possible effects due to the yoghurt "vehicle", as well as other environmental influences. Sunflower oil was included in the yoghurt of another group to control for the increased energy and linoleic acid intake from lecithin, because an intake of 20g SternpurPM lecithin provides 622 kJ (148kcal) and contains 59% linoleic acid (Stern typisch Lecithin & Soja, Hamburg, Germany). After two baseline fasting blood samples (one week apart) and anthropometric measurements (height, weight) were obtained, the subjects started with the treatment. The means of the two baseline values were calculated. Fasting blood samples and anthropometric measurements were then taken after 2 and 4 weeks of treatment. BMI (kg/m^2) was calculated.

The subjects were asked to maintain their usual diet, alcohol consumption and physical activity. One of the subjects in the lecithin group were excluded from the analysis, because he did not maintain his usual lifestyle. He went on vacation and gained 7kg.

Table 1 Fatty acid composition (g) of 175g of frozen yoghurt, without or with lecithin or sunflower oil

Nutrient	Frozen yoghurt#	Frozen yoghurt# with 20g Sternpur PM lecithin*	Frozen yoghurt with 17g sunflower oil#
Energy (kJ)	948	1461	1485
Fatty acids (g):			
Palmitic acid (C16:0)	0.84	4.74	1.78
Stearic acid (C18:0)	0.30	1.06	1.12
Oleic acid (C18:1, n-9)	0.70	2.62	3.69
Linoleic acid (C18:2, n-6)	0.04	11.84	10.92
α-Linolenic acid (C18:3, n-3)	0.04	1.43	0.21
Phospholipids (%)	-	96	-

* Composition as given by Stern typisch Lecithin & Soja, Hamburg, Germany

\# Composition as given by South African Food Composition Tables (Gouws & Langenhoven, 1986; Langenhoven *et al*, 1991)

Blood samples

The subjects were required to fast overnight (12 h). Venous blood samples were collected using a 21-gauge scalp vein infusion set. All samples were drawn with minimal stasis and between 07:00 and 10:00 to avoid effects of diurnal variation. EDTA blood was used for the analysis of haemotocrit and haemoglobin. Clotted, citrated and EDTA blood were centrifuged for 15 minutes at 2000*g* to yield serum for lipoprotein, plasma for haemostatic and plasma for fatty acid assays, respectively. Serum and plasma were divided into aliquots and frozen at -72°C within 30 minutes for later analysis.

Experimental methods

To monitor compliance, the fatty acid composition of total plasma lipids was determined with chloroform/methanol extractions as described by Smuts *et al* (1992) (Coefficient of variance (CV) = 4%). TC concentrations were determined enzymatically with the Technicon Omnipak-method ((SM-4-0139/86), Bayer, Puteaux Cedex). LDL-C was determined with the CHOD-Iodide method (Merck, Darmstadt, Germany). LDL was precipitated by heparin at its iso-electric point (pH 5.12). After centrifugation, the HDL-C and the very low density lipoprotein cholesterol remained in the supernatant and were determined with enzymatic methods. The LDL concentration was then calculated according to the following formula: LDL-C = TC - cholesterol in supernatant. Apoprotein (apo) A and apoB were determined with an immuno-chemical method (Behring Institute, Marburg, West Germany, antiserum for human apoA (code no. OUED), and human apoB (code no. OSAN)). Lipoprotein (a) (Lp(a)) was determined with a radio-immunological method (Pharmacia Apo(a) RIA). Plasma fibrinogen was measured with the method of Ratnoff & Menzies (1951) (CV = 3.31%) that measures the concentration of clottable protein. MPC was measured by precipitating thrombin unclottable proteins with protamine sulfate (Sigma, St. Louis, USA, Cat. no. p-4020) (Lipinski *et al*, 1995) and then measuring the protein concentration with the method of Ratnoff & Menzies (1951) (CV = 3.6%). Haematocrit was determined with the capillary tube method and haemoglobin with a calorimetric method (Boehringer Mannheim, Cat.no. 124729).

Statistical analysis

Because a normal distribution of data could not be assumed, significant differences within groups were determined with the Wilcoxon matched pairs test and between groups with the Mann-Whitney U test. The computer software package Statistica® was used for these analyses. 95% Confidence intervals (CI) were calculated (Conover, 1971). All data are presented as median (95% CI). A *P*-value of less or equal to 0.05 was regarded as being statistically significant.

Results

The median (95% CI) levels of the variables during yoghurt, lecithin and sunflower oil treatments are summarised in Tables 2-5.

Table 2 indicates that plasma linoleic acid (C18:2) levels increased significantly with both lecithin and sunflower oil treatments and were also significantly higher after 2 weeks of treatment, compared to the yoghurt group. Plasma palmitic (C16:0) and palmitoleic acid (C16:1) levels increased significantly in the yoghurt group. After 4 weeks of treatment the median palmitic acid level was significantly higher compared to the lecithin and sunflower oil groups. Plasma stearic acid (C18:0) and oleic acid (C18:1) levels did not change with any of the treatments while plasma gamma-linolenic acid (C18:3, n-6) levels decreased significantly with sunflower oil treatment and alpha-linolenic acid (C18:3, n-3) levels increased with lecithin treatment.

Weight and BMI was not affected in the yoghurt and lecithin groups, but increased significantly with sunflower oil treatment. Haemoglobin levels tended to decrease in the lecithin and sunflower oil groups and the decrease was significant in the yoghurt group. These small decreases were, probably, not of any clinical significance, because reference values for haemoglobin in men are 8.68-11.16 mmol/L (Lee & Nieman, 1993). Haematocrit levels did not change with any of the treatments (Table 3).

Table 2 Dietary compliance: median [95% confidence interval (CI)] changes in plasma fatty acids (% w/w) during treatment

		Yoghurt		Yoghurt with Lecithin		Yoghurt with Sunflower oil	
		Median	95%CI	Median	95%CI	Median	95%CI
C16:0	B	21.06*	16.87-24.37	21.47	17.67-28.73	21.47	20.05-25.52
	M	22.70	20.10-24.64	22.33	17.65-24.72	21.20	18.33-23.10
	E	22.98*#●	21.17-29.13	20.90#	17.71-25.15	21.34●	17.71-26.05
C16:1	B	1.54*	0.86-2.24	1.72	1.20-2.22	1.41	0.92-2.26
	M	1.66	0.74-2.12	1.52	0.80-2.42	1.73	0.91-2.63
	E	1.95*	0.91-4.32	1.71	0.98-3.04	1.50	1.05-2.23
C18:0	B	7.64	7.21-8.90	7.68	6.79-8.89	7.55	6.87-8.90
	M	7.56	6.87-8.65	7.32	6.82-7.99	7.16	6.86-8.25
	E	7.64	6.91-7.96	7.52	7.24-8.00	7.32	6.57-8.55
C18:1	B	23.72	20.44-25.74	22.35	19.63-24.20	22.99	16.28-27.35
	M	22.86	19.34-27.34	21.68	18.21-25.12	19.95	19.08-24.75
	E	22.77	21.73-26.79	20.59	18.82-25.13	21.68	15.37-24.97
C18:2	B	32.13	29.48-33.09	32.87*♣	25.46-38.71	31.44♥	27.83-43.33
	M	31.59#●	28.20-33.92	34.59#♣	31.37-40.09	35.55●	31.58-41.64
	E	30.19	24.91-33.28	35.70*	29.23-39.53	36.27♥	30.44-45.96
C18:3 (n-6)	B	0.50	0.31-0.75	0.49	0.33-0.65	0.53*	0.29-0.69
	M	0.49	0.42-0.88	0.50	0.41-0.67	0.49	0.30-0.97
	E	0.63	0.44-0.99	0.58	0.40-0.71	0.49*	0.32-1.07
C18:3 (n-3)	B	0.32	0.22-0.43	0.30*#	0.23-0.56	0.29	0.20-0.75
	M	0.43	0.22-0.63	0.54*	0.30-0.71	0.35	0.24-0.47
	E	0.35	0.23-0.63	0.52#	0.25-0.80	0.26	0.21-0.60

B: Baseline; M: Middle; E: End

Medians (95% CI) with the same symbol differed significantly ($p \leq 0.05$)

Table 3 Median [95% confidence interval (CI)] age and changes in anthropometric, blood pressure, haematocrit and haemoglobin levels during treatment

		Yoghurt		Yoghurt with Lecithin		Yoghurt with Sunflower oil	
		Median	95%CI	Median	95%CI	Median	95%CI
Age (years)		44.00	30-58	47.50	34-56	58.00	31-63
Weight (kg):	B	78.70	72.2-108.0	83.95	79.6-86.8	86.60*	67.6-100.1
	M	79.20	71.6-108.0	84.10	79.6-87.4	88.20*	68.2-100.0
	E	78.80	71.6-107.5	83.90	79.6-88.6	88.80	68.4-100.5
BMI (kg/m^2):	B	26.20	22.91-31.22	26.39	25.36-28.15	27.64*#	22.85-33.01
	M	25.98	23.17-31.22	26.39	25.54-28.28	28.15*	23.05-33.28
	E	25.98	23.36-31.07	26.19	25.63-28.21	28.26#	23.12-32.81
Haematocrit	B	47.00	43.50-49.00	44.75	40.00-45.50	45.00	41.00-46.50
(%)	M	48.00	44.00-52.00	45.00	40.00-47.00	47.00	36.00-50.00
	E	48.00	43.00-50.00	44.00	43.00-47.00	47.00	40.00-48.00
Haemoglobin	B	11.36*	10.54-12.45	11.63	10.54-12.18	11.63	8.91-12.18
(mmol/L)	M	11.91	10.00-13.00	11.49	7.00-12.18	11.63	7.82-12.18
	E	10.82*	10.27-11.91	10.68#	10.27-11.36	10.00#	9.18-11.91

B: Baseline; M: Middle; E: End; BMI: Body mass index

Medians (95% CI) with the same symbol differed significantly ($p \leq 0.05$)

As shown in Table 4, lecithin therapy had no effect on serum TC, TG, HDL-C, LDL-C, LDL-C/HDL-C or apoA levels. Serum apoB and Lp(a) levels increased significantly after 2 weeks of lecithin treatment, but these levels returned to baseline levels after 4 weeks of treatment. Serum TC levels increased significantly in the group who received sunflower oil. HDL-C levels decreased and LDL-C/HDL-C ratio increased significantly in the yoghurt group, but these changes were probably not of any clinical significance.

Plasma fibrinogen and MPC levels were not effected by yoghurt or lecithin treatments, but MPC levels were significantly reduced by sunflower oil treatment (Table 5).

Table 4 Median [95% confidence interval (CI)] changes in serum lipoprotein levels during treatment

		Yoghurt		Yoghurt with Lecithin		Yoghurt with Sunflower oil	
		Median	95%CI	Median	95%CI	Median	95%CI
TC (mmol/L)	B	6.40	5.32-7.62	6.34	6.12-8.03	6.76*	5.90-6.80
	M	6.52	5.73-7.78	6.82	6.22-8.41	6.78	6.03-7.50
	E	6.53	5.99-7.98	6.58	5.35-8.36	6.90*	6.08-7.12
TG (mmol/L)	B	1.93	1.43-3.15	2.13	0.96-6.05	1.99	1.06-4.56
	M	1.67	1.38-1.86	1.56	1.26-1.68	1.61	1.44-1.95
	E	1.99	1.66-3.82	1.72	1.16-2.48	1.59	0.99-2.27
HDL-C	B	1.13*	0.95-1.36	1.12	0.95-1.49	1.05	1.00-1.48
(mmol/L)	M	1.05	0.86-1.27	1.15	0.83-1.72	1.12	1.94-1.30
	E	1.07*	0.81-1.21	1.19	0.75-1.68	1.05	0.91-1.53
LDL-C	B	4.99	3.99-6.07	4.91	4.22-6.53	5.25	4.44-5.49
(mmol/L)	M	5.02	4.43-6.29	5.03	4.56-6.85	5.15	4.40-5.97
	E	4.96	4.44-6.56	4.98	3.72-6.28	5.19	4.68-5.79
LDL-C/	B	4.63*	3.36-5.29	4.47	2.88-6.87	5.00	3.39-5.34
HDL-C	M	4.77	4.47-5.99	4.54	2.80-8.25	4.30	3.52-6.35
	E	5.26*	4.15-6.13	4.38	2.21-8.37	5.07	3.40-6.36
ApoA	B	1.48	1.15-1.74	1.37	1.30-1.60	1.35	1.22-1.70
(mmol/L)	M	1.38	1.16-1.55	1.42	1.25-1.85	1.36	1.18-1.76
	E	1.40	1.18-1.51	1.47	1.27-1.65	1.34	1.10-1.70
ApoB	B	1.32	1.09-1.53	1.32*	1.17-1.72	1.36	1.18-1.52
(mmol/L)	M	1.35	1.14-1.59	1.40*#	1.23-1.86	1.40	1.17-1.51
	E	1.32	1.25-1.65	1.31#	1.10-1.70	1.40	1.12-1.51
Lp(a) (U/L)	B	200.5	17.0-944.2	202.3*	17.5-794.0	157.0	58.0-671.0
	M	194.5	13.9-431.3	241.3*	16.4-861.8	151.2	54.6-635.5
	E	153.0	14.7-453.6	213.4	9.64-324.2	173.0	63.4-670.3

B: Baseline; M: Middle; E: End; TC: Total cholesterol; TG: Triglyceride; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; Apo: Apoprotein; Lp(a): Lipoprotein (a)

Medians (95% CI) with the same symbol differed significantly ($p \leq 0.05$)

Table 5 Median [95% confidence interval (CI)] changes in plasma fibrinogen and macromolecular protein complex (MPC) during treatment

		Yoghurt		Yoghurt with Lecithin		Yoghurt with Sunflower oil	
		Median	95%CI	Median	95%CI	Median	95%CI
Fibrinogen (g/L)	B	2.88	1.14-3.47	3.16	1.83-4.00	3.11	1.49-4.07
	M	2.59	1.09-3.69	3.13	1.72-4.84	3.13	1.75-4.75
	E	2.40	2.16-5.44	3.68	1.69-4.38	2.56	1.66-4.00
MPC (g/L)	B	5.03	3.05-6.39	5.30	2.26-16.75	5.30*	3.27-11.16
	M	4.88	2.65-5.76	6.14	2.04-12.68	4.63	2.74-8.84
	E	4.57	2.99-8.05	5.58	1.89-7.74	3.81*	2.84-10.43

B: Baseline; M: Middle; E: End

Medians (95% CI) with the same symbol differed significantly ($p \leq 0.05$)

Discussion

The significant increases in plasma linoleic acid levels with both lecithin and sunflower oil treatments indicate good compliance to the treatments. Because the lecithin product also contains 7% w/w of alpha-linolenic acid (Stern Lecithin & Soja GmbH & Co Kg, Hamburg, Germany) the significant increase in plasma alpha-linolenic acid levels may also be indicative of good compliance to the lecithin treatment. Plasma palmitic and palmitoleic acid levels increased significantly in the control group. These more saturated fatty acids (SFA) might have been synthesised in the body from an increased intake of carbohydrate (Lands, 1995), because it has been indicated that the SFA composition in the plasma is not a good indicator of dietary intake of SFA (Simon *et al*, 1995). The significant decrease in gamma-linolenic acid levels with sunflower oil treatment is probably of no clinical significance since the changes in the yoghurt and lecithin groups were even greater although not significant.

The salient observations of this study on free living hyperlipidaemic men were that lecithin had no effect on the measured risk factors and markers for coronary heart disease but that the sunflower oil resulted in increased body weight, serum TC and reduced plasma MPC levels. The increased serum TC levels may probably be explained by the increase in body weight in these subjects (Newman *et al*, 1990).

Results from clinical trials of lecithin have reported TC and LDL-C lowering effects (Davies & Murdoch, 1959; Morrison, 1958; Saba *et al*, 1978; Steiner & Domanski, 1944; Tompkins & Parkin, 1980) while other studies showed no significant effects (Childs *et al*, 1981; Cobb *et al*, 1980; Greten *et al*, 1980; Kesaniemi & Grundy, 1986; Ter Welle *et al*, 1974). A decreasing effect of lecithin on TG levels (Cobb *et al*, 1980; Saba *et al*, 1978; Tompkins & Parkin, 1980) and in some studies no effect (Childs *et al*, 1981; Greten *et al*, 1980; Kesaniemi & Grundy, 1986; Ter Welle *et al*, 1974) have been reported. Some studies reported no effect of lecithin on HDL-C levels (Greten *et al*, 1980; Kesaniemi & Grundy, 1986; Prack *et al*, 1983), while Childs *et al* (1981) reported an increasing effect on HDL-C, independent of its polyunsaturated fatty acid moiety. As discussed in the introduction most of these studies were poorly designed and are not comparable to the present study because of the different study designs, different amounts of lecithin used, ranging from 0.7 to 54 g/d (Knuiman *et al*, 1989), and different concentrations of phospholipids in lecithin products, ranging from 29% (Childs *et al*, 1981) to 96% in the present study.

It has been demonstrated in humans that intake of lecithin (Kesaniemi & Grundy 1986) and infusion of lecithin intraduodenally (Beil & Grundy, 1980), decreases the absorption of cholesterol in the small intestine. Kesaniemi & Grundy (1986) suggested that if lecithin is given in multiple doses throughout the day, the inhibition of cholesterol absorption might be higher and that the amount of dietary cholesterol may also have an effect on the results. This may, in part, explain why lecithin did not lower TC levels in this particular study, because these subjects followed a low cholesterol diet. Other studies in which subjects followed a low cholesterol baseline diet could also not demonstrate TC lowering with lecithin (Greten *et al*, 1980; Kesaniemi & Grundy, 1986).

In this study lecithin treatment had no effect on plasma fibrinogen or MPC levels, but sunfloweroil treatment decreased MPC levels significantly that may probably decrease thrombotic risk (Lipinski *et al*, 1995). This possible beneficial effect of sunflower oil on MPC needs to be further investigated.

Conclusion

Lecithin treatment had no independent effects on serum lipoprotein, plasma fibrinogen or MPC levels in free living hyperlipidaemic men in a double-blind study which controlled for possible indirect effects.

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CHAPTER 6

HIGH FIBRE *VS* LOW FIBRE MEALS AND POSTPRANDIAL SERUM LIPOPROTEIN AND PLASMA FACTOR VII COAGULANT ACTIVITY ($FVII_C$) RESPONSES

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ABSTRACT

The objectives of this study were to determine what the effects of high fibre *vs* low fibre meals would be on postprandial serum lipoprotein and factor VII coagulant activity ($FVII_c$) responses. Although most of the time is spent in the postprandial state, a state that can be atherogenic, limited data on the effects of diet, especially dietary fibre, on postprandial lipoproteins are available. $FVII_c$ was measured as it's activation is one of a number of mechanisms through which dietary-derived lipids can influence atherogenesis. Seven healthy, normolipidaemic men received either a high fibre or low fibre test meal, in the form of muffins, in random order, on two separate days, one to two weeks apart. The high fibre meal provided 17.4g fibre, mainly wheat bran. Blood samples were obtained at 0, 2, 3, 4, 6 and 8 hours after ingestion of the meal. Serum triglycerides (TG), insulin, total cholesterol, high density lipoprotein cholesterol (HDL-C), plasma retinyl palmitate and $FVII_c$ were measured with standard methods. The high fibre meal caused more gradual serum TG, insulin and plasma retinyl palmitate response curves *vs* the low fibre meal, resulting in significantly higher levels at 3 hours after the test meal. HDL-C levels were significantly lower at 3 hours after the high fibre meal compared to the low fibre meal. Plasma $FVII_c$ levels did not change significantly from baseline for either of the two test meals. It is concluded that high fibre muffins caused more gradual serum TG and retinyl palmitate response curves compared to low fibre muffins resulting in significantly higher levels and lower HDL-C levels at 3 hours. With the intake of normal physiological fat amounts, as used in this study (30.5g fat), factor VII is not activated even if serum TG increases.

SHORT TITLE: Dietary fibre and postprandial lipaemia and factor VII_c.

KEY WORDS: postprandial lipaemia, postprandial factor VII, dietary fibre

INTRODUCTION

The postprandial state, defined as the time period between food ingestion and 6-8 hours thereafter (Patsch *et al.* 1983) is characterised by the movement of nutrients from the lumen of the intestines into the circulation. Zilversmit (1979) proposed that the remnant particles formed from dietary-derived lipids are atherogenic. Several studies have demonstrated that postprandial levels of intestinal triglyceride (TG)-rich lipoproteins are higher in those individuals with coronary heart disease (CHD) and that these increased levels are not dependent on patients having an abnormal fasting TG-status (Cohn, 1998; Karpe, 1997; Steiner, 1993). There is, however, a complete lack of prospective studies to confirm this relationship (Karpe, 1997). A number of possible mechanisms have been proposed through which the increased presence of TG-rich lipoproteins in the circulation can influence atherogenesis. The first involves lipoprotein remodelling where TG are transferred from chylomicrons to low density lipoprotein (LDL) and high density lipoprotein (HDL) particles in exchange for cholesterol esters (CE) through lipid transfer proteins (Lechleitner *et al.* 1994; Zilversmit, 1995). Thus, chylomicrons, while shedding their TG, acquire larger amounts of CE resulting in smaller, atherogenic chylomicron remnant particles. TG transferred to LDL and HDL are susceptible to hydrolysis by hepatic lipase (HL), which reduce the size of these lipoproteins resulting in small, more atherogenic LDL particles, in decreased HDL_2, the anti-atherogenic particle, and increased HDL_3 particles (Lechleitner *et al.* 1994; Zilversmit, 1995). The second mechanism proposed is that chylomicron remnant particles can be taken up by macrophages through phagocytosis and produce foam cells independently of prior oxidation or intracellular cholesterol concentration (Bradley & Gianturco, 1994; Mamo *et al.* 1997; Van Lenten *et al.* 1985). The third is that TG-rich particles may be directly damaging to the endothelium via oxidative mechanisms (Sattar *et al.* 1998). Fourthly, increased postprandial TG levels are associated with increased factor VII coagulant activity ($FVII_c$) (Marckmann *et al.* 1993; Miller *et al.* 1991; Negri *et al.* 1993; Silveira *et al.* 1994), a known risk factor for CHD (Assman *et al.* 1996; Heinrich *et al.* 1994; Meade *et al.* 1980; Meade *et al.* 1986). Factor VII, after forming a complex with tissue factor, is the initiating step for the activation of the extrinsic coagulation pathway that will lead to the formation of fibrin clots (Murphy, 1994). $FVII_c$, the classical assay most often used to measure factor VII, reflects the activated part of factor VII in the blood as well as inactivated factor VII (factor VII zymogen) that becomes activated during the assay (Mennen *et al.* 1996). Since the greatest part of the day is spent in

postprandial state, particularly when eating patterns involve three meals a day, it is important that the effects of diet on postprandial lipoproteins are examined. A large body of literature on the effect of diet on fasting lipoprotein levels is available, but data on postprandial lipoproteins are limited, especially on the effects of dietary fibre. A few studies have examined the effect of soluble dietary fibre (especially oat bran) on postprandial lipoproteins, with controversial results (Cara *et al.* 1992; Dubois *et al.* 1995; Dubois *et al.* 1996; Redard *et al.* 1990). In the one study that could be found which examined the effect of wheat bran, lower postprandial serum TG and chylomicron TG responses compared to those after a test meal were reported (Cara *et al.* 1992).

The objectives of this study were to determine what the effect of wheat bran, incorporated into a baked product, would be on the postprandial lipoprotein responses and whether these responses will alter the $FVII_c$. The high fibre baked product, a muffin, was developed in our laboratory (Bosman *et al.* 1996) for use in high fibre diets.

MATERIALS AND METHODS

Subjects

The study was approved by the Ethics Committee of the Potchefstroom University for Christian Higher Education (Ethics no. HHK1M5-94). An informed consent form was signed by each of the seven healthy, normolipidaemic, young men (mean ± standard deviation (SD) age of 19.71±1.70 years) who voluntarily participated in this study.

The exclusion criteria for selection of subjects were: smoking, obesity, diabetes, hyperlipidaemia and any medication that may influence the lipoprotein metabolism. Mean ± SD baseline body mass index (21.91±2.5kg/m^2), fasting total serum cholesterol (TC) (4.36±0.43mmol/L), TG (0.65±0.20mmol/L), high density lipoprotein cholesterol HDL-C (0.94±0.14mmol/L), glucose (4.27±0.77mmol/L), insulin (14.62±4.8µIU/mL) and plasma $FVII_c$ (70.43±16.31%) levels were within normal ranges. Body weights did not vary during the study.

Study design

Two meals consisting of either eight high fibre or six low fibre muffins together with 100 000 IU of vitamin A (Arovit, H/22/2241, Roche, Johannesburg, South Africa) were consumed in random order on two separate days one to two weeks apart. Each subject thus served as his own control. The nutrient composition of the two meals are summarised in Table 1. The type of fibre used was wheat bran.

Table 1. *The nutrient composition of the test meals**

Nutrient	High fibre muffins (280g)	Low fibre muffins (210g)
Energy (kJ)	2887.4	2839.7
Protein (g)	23.8 (14%E)	23.8 (14%E)
Total fat (g)#	30.5 (40%E)	30.1 (40%E)
Cholesterol (mg)	63.8	61.0
Carbohydrate (g)	77.7 (46%E)	78.3 (46%E)
Total fibre (g)	17.4##	1.2

* Composition as given by the South African Food Composition Tables (Langenhoven *et al.* 1991).
\# Mainly from sunflower oil
Mainly from wheat bran
%E: Percentage of the total energy intake

Measurement of plasma retinyl palmitate (vitamin A) is used as marker for the presence of lipoproteins of intestinal origin (chylomicrons and chylomicron remnants) in the circulation. The rationale for this approach is based on the concept that dietary vitamin A is absorbed with chylomicron particles in the intestine in its retinyl ester form, predominantly retinyl palmitate. Retinyl esters remain within the chylomicron until the remnant particle is removed by the liver (summarised by Blomhoff, 1994; Cohn *et al.* 1993; Krasinski, *et al.* 1990 and O'Meara, *et al.* 1992). There are some concerns regarding the validity of the use of plasma retinyl palmitate as marker for intestinally-derived lipoproteins because plasma retinyl esters have also been found in LDL and HDL (Krasinski, *et al.* 1990). However, the transfer of retinyl esters to other circulating lipoproteins have been reported to be insignificant (Berr & Kern, 1984) and may not occur before at least nine hours (Krasinski *et al.* 1990). Thus, for this study that lasted eight hours, plasma retinyl palmitate was a useful marker for intestinal-derived lipoproteins.

The subjects were instructed not to consume any alcohol 72 hours before, or to do any strenuous exercise 24 hours before the test periods. To avoid any metabolic effects because of previous food intake (Wolever *et al.* 1988) all the subjects had the same dinner at 19h00 the evening preceding the test period. This meal consisted of three slices of brown bread with 15g margarine and 90g cheese, an apple and coffee or tea.

On the morning of the test period, after a 12 hour overnight fast, anthropometric measurements and blood samples were taken. The test meal with coffee or tea, without milk or sugar, was then consumed within 20 minutes. The vitamin A tablets were taken directly after the meal. During the postprandial period, subjects were food-restricted but were allowed to consume water *ad libitum.* Blood samples were obtained at 2, 3, 4, 6 and 8 hours after ingestion of the meal.

The subjects were asked to maintain their usual diet, alcohol consumption and physical activity for the duration of the study.

Blood samples

Venous blood samples were collected by using a cannula vein infusion set (0.1mm/20 GOD x 33ml, Omni-med) that stayed in the vein for the duration of the test period. Before blood was drawn the first 2ml blood was discarded. Clotted, citrated and EDTA blood were centrifuged for 15 min at 2000 *g* to yield serum for the analysis of lipoprotein and insulin levels, plasma for $FVII_c$ and EDTA plasma for retinyl palmitate levels, respectively. Serum and plasma were divided into aliquots and frozen at -72°C within 30 min for later analysis.

Experimental methods

TC (coefficient of variance {CV}=4.14%), TG (CV=3.6%) and HDL-C (CV=0.89%) were determined enzymatically with test kits from Boehringer Mannheim (Mannheim, West Germany). Serum insulin was determined with a radio-immunologic method (Medgenix Diagnostics, Belgium) (CV=6.2%) and plasma retinyl palmitate by high pressure liquid chromatography (CV=13.8%). Plasma $FVII_c$ was determined with a one-stage clotting assay using an ACL-200 automated coagulation analyser, calcium thromboplastin extracted from rabbit brain (PT-Fib kit, Instrumentation Laboratories {IL}, Milan, Italy, Cat.no. 84682-10) and factor VII deficient plasma (IL, Milan, Italy, Cat.no. 84662-50) (CV=2.49%).

Statistical analysis

The computer software package Statistica® was used. Significant differences between test meals at specific times were determined with the Wilcoxon matched pairs test. The MANOVA test was used to compare postprandial values with baseline values. Values over time are expressed as variations of median concentrations over baseline (fasting baseline values being zero). A *P*-value of less or equal to 0.05 was regarded as being statistically significant.

RESULTS

The median postprandial responses of the variables to the two test meals are illustrated in figures 1-6.

Serum TG levels (Fig. 1) rose significantly from baseline for both the test meals, peaked at 2 hours, remained significantly increased until 4 hours after which they decreased to baseline before 8 hours. The decrease after 2 hours was more rapid for low fibre muffins than for high fibre muffins, causing a significant difference at 3 hours.

Plasma retinyl palmitate levels (Fig. 2) rose significantly from baseline and remained significantly elevated for the duration of the test period. The response curve for low fibre muffins peaked at 2 hours and for high fibre muffins at 3 hours, causing a significantly lower median level for low fibre muffins compared to high fibre muffins at 3 hours.

The serum insulin response curves (Fig. 3) of the two test meals appeared very different. For low fibre muffins the serum insulin levels peaked, not significantly (probably because of great individual variation), at 2 hours and then decreased very rapidly to significantly below baseline at 3 hours and remained below baseline. For high fibre muffins, the serum insulin levels peaked at 2 hours and then, very gradually, decreased to below baseline at 6 hours. The median levels after low fibre muffins were significantly lower at 3 and 4 hours compared to high fibre muffins.

Total serum cholesterol levels (Fig. 4) decreased significantly for both test meals. For low fibre muffins statistical significance from baseline was reached at 3 hours and for high fibre muffins at 2, 3, 4 and 8 hours. The responses to the two test meals did not differ significantly.

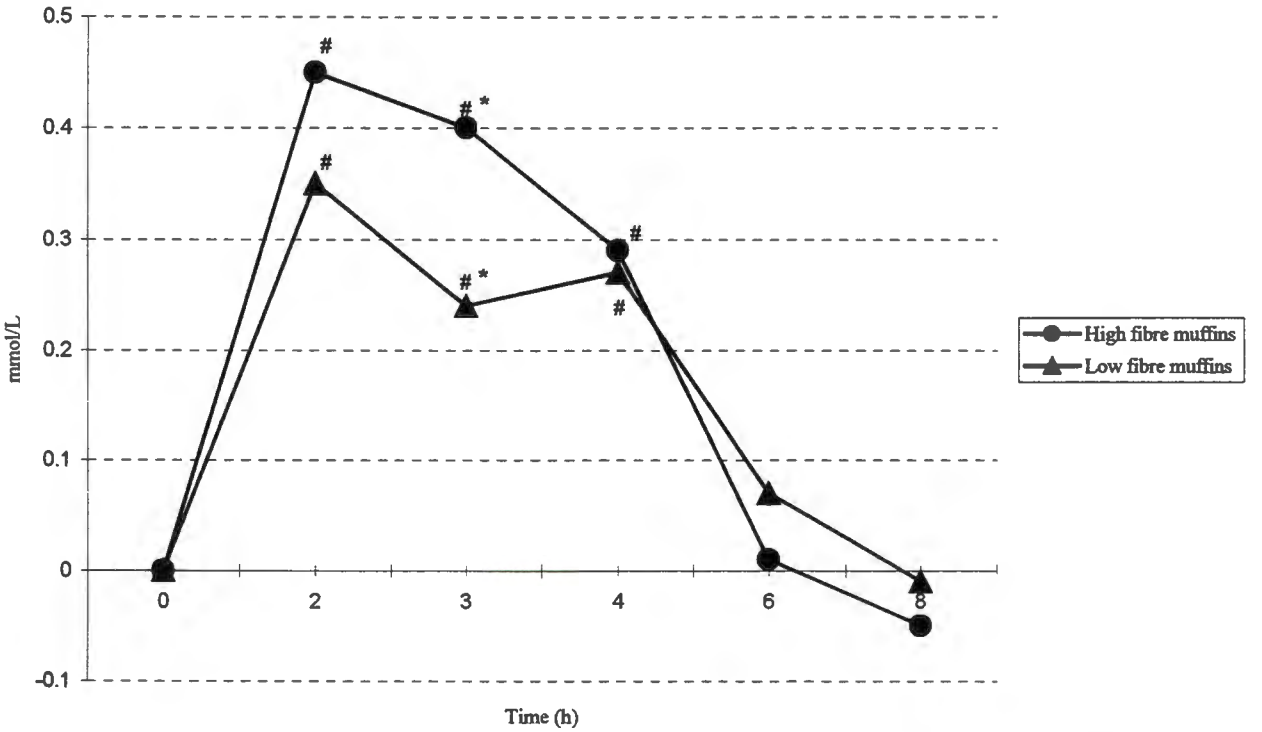

Fig. 1. Median serum triglyceride responses to the test meals. (#: Significantly different from baseline; *: Significant diffences between test meals at specific times).

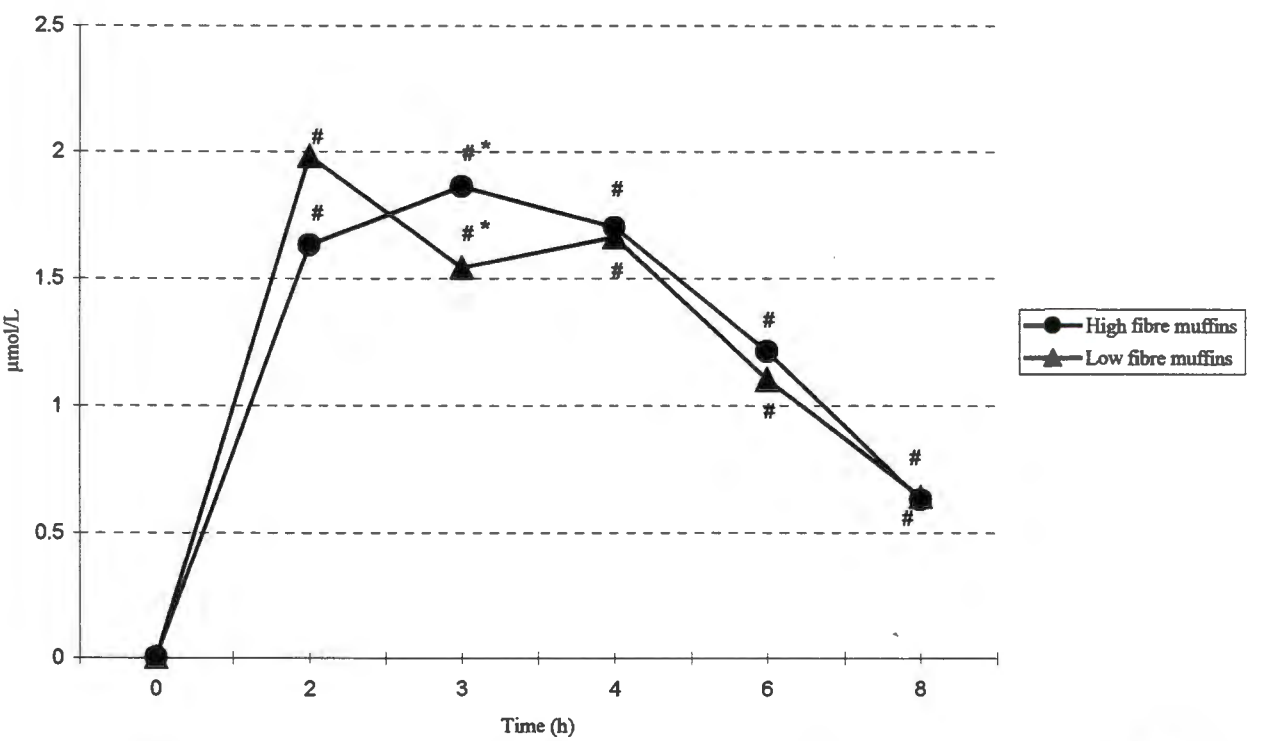

Fig. 2. Median plasma retinyl palmitate reponses to the test meals. (#: Significantly different from baseline; *: Significant diffences between test meals at specific times).

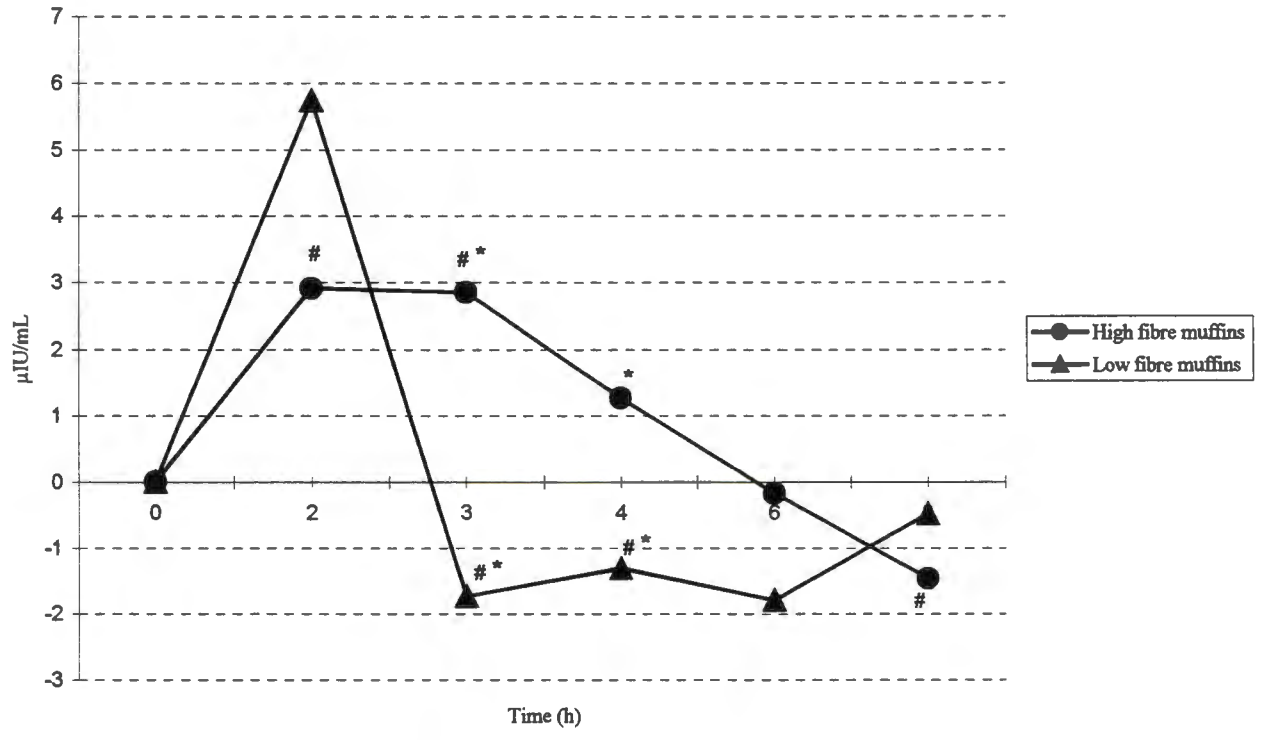

Fig. 3. Median serum insulin reponses to the test meals. (#: Significantly different from baseline; *: Significant diffences between test meals at specific times).

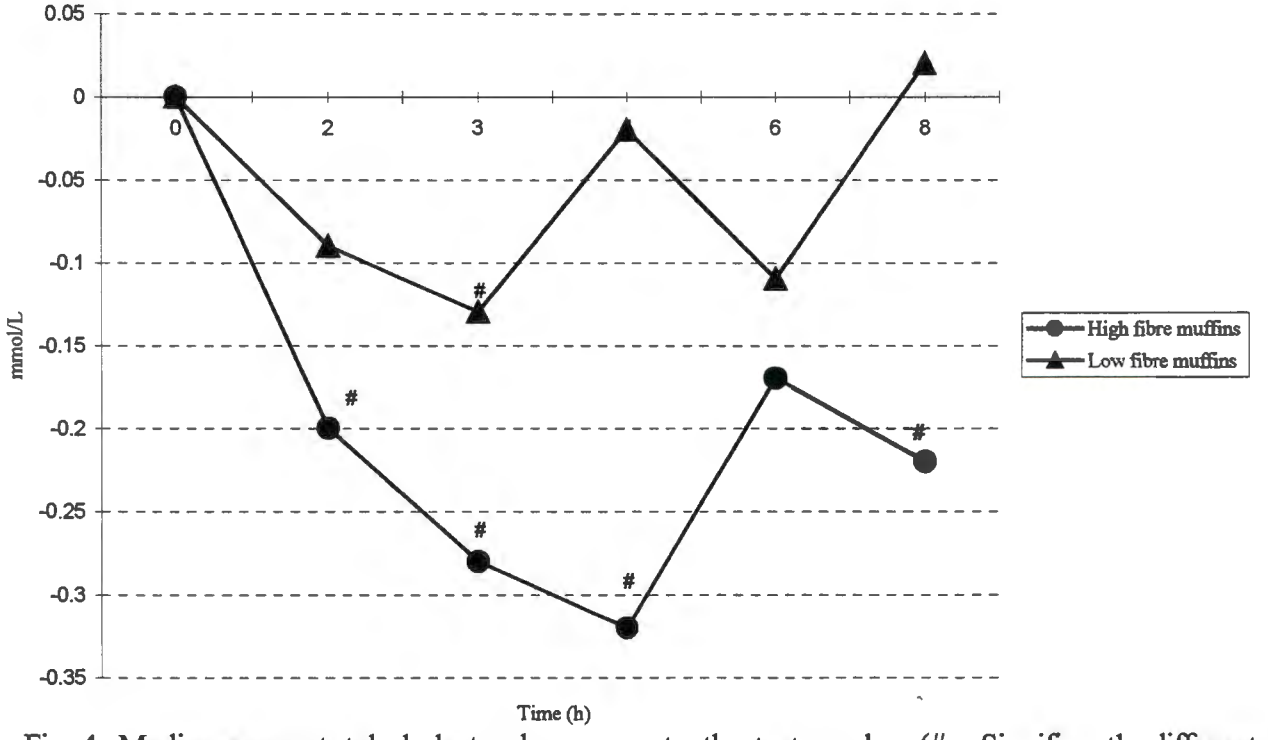

Fig. 4. Median serum total cholesterol reponses to the test meals. (#: Significantly different from baseline).

The serum HDL-C response curves of the two test meals also differed (Fig. 5). For low fibre muffins serum HDL-C levels decreased significantly from baseline and reached the lowest level at 2 hours after which it rose again and reached baseline levels before 8 hours. For high fibre muffins the serum HDL-C levels decreased significantly from baseline and reached the lowest level after 3 hours after which it gradually increased and nearly reached baseline levels within 8 hours. At 3 hours the median serum HDL-C level was significantly higher for the low fibre muffins than the high fibre muffins.

The plasma $FVII_c$ levels (Fig. 6) did not change significantly from baseline for either of the two test meals and the two test meals also did not differ.

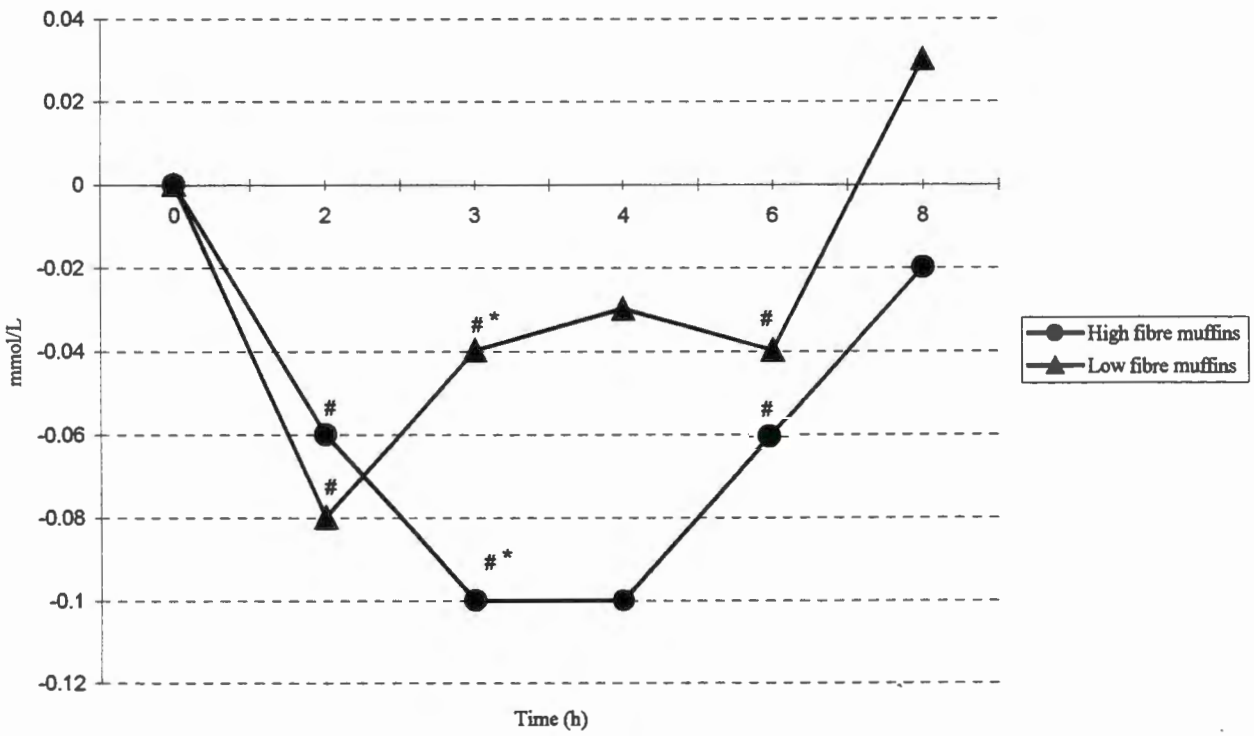

Fig. 5. Median serum high density lipoprotein cholesterol (HDL-C) reponses to the test meals. (#: Significantly different from baseline; *: Significant diffences between test meals at specific times).

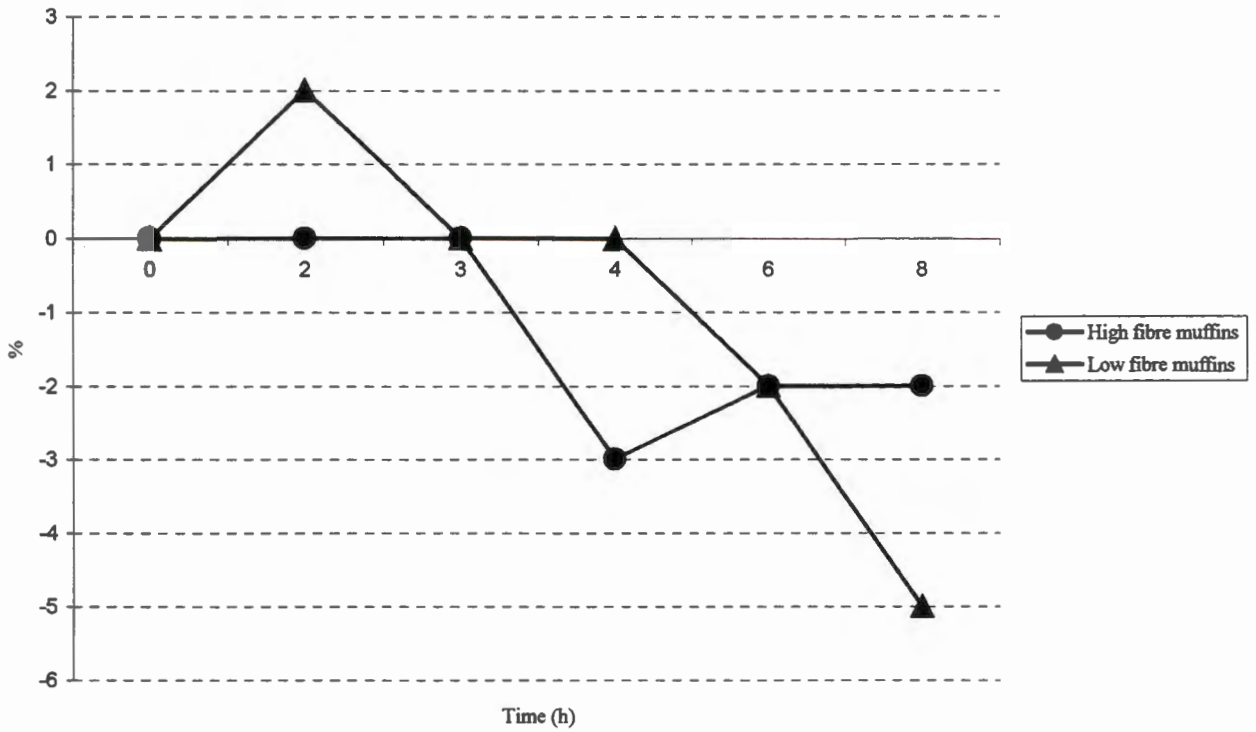

Fig. 6. Median plasma factor VII coagulant activity responses to the test meals.

DISCUSSION

The salient observations of this study in which the postprandial serum lipoprotein reposes of high fibre (wheat bran) and low fibre muffins were compared, and the effect of these responses on the activation of factor VII, were that:

1) Although the overall responses of serum TG, plasma retinyl palmitate and serum HDL-C did not differ between the two test meals, high fibre muffins caused more gradual serum TG and plasma retinyl palmitate response curves than low fibre muffins, resulting in higher serum TG and plasma retinyl palmitate and lower HDL-C levels at 3 hours after the test meal.

2) $FVII_c$ levels was not significantly altered from baseline for either of the two test meals even though serum TG levels increased significantly.

Very few studies have examined the effect of dietary fibre, especially wheat bran (insoluble dietary fibre), on postprandial lipoproteins. Anderson *et al.* (1995) examined the effects of

high fibre (26.1g total fibre, 13.2g soluble fibre) *vs* low fibre meals and found that although the serum TG responses did not differ significantly, values were significantly higher at 2 and 3 hours after a high fibre meal. Results from studies that examined the effects of soluble fibre (especially oat bran and guar gum) are controversial. It has been shown that soluble fibre can decrease (Cara *et al.* 1992) or increase (Dubois *et al.* 1995; Dubois *et al.* 1996; Redard *et al.* 1990) postprandial TG responses compared to low fibre test meals. The one study that could be found in which the effect of insoluble fibre (wheat fibre) on postprandial lipaemia was examined, demonstrated lower postprandial serum TG and chylomicron TG repines curves with a high wheat fibre compared to a control test meal (Cara *et al.* 1992). In contrast with the study of Cara *et al.* (1992), this study showed that the high fibre muffins caused more gradual serum TG and plasma retinyl palmitate (marker for chylomicrons and their remnants) response curves than low fibre muffins, resulting in significantly higher values at 3 hours compared to low fibre muffins. This may be explained by two possible mechanisms:

Firstly, the rate of chylomicron catabolism and removal might have been slowed by the high fibre muffins. Insulin stimulates the synthesis of lipoprotein lipase (Meyer, 1988), the enzyme responsible for the hydrolysis of TG in chylomicrons (Mann & Skeaff, 1998). The rapid increase and then decrease of serum insulin after low fibre muffins might indicate initial accelerated TG clearance from the circulation, whereas the more gradual secretion of insulin after high fibre muffins may indicate a more gradual and slower clearance.

Secondly, high fibre muffins might have caused a delayed entry of chylomicrons into the circulation. It has been shown that the plasma peak of retinyl palmitate may occur 1-3 hours after TG, due to delayed absorption of vitamin A relative to lipid (Karpe & Hamsten, 1995; Krasinski *et al.* 1990). In this study, the retinyl palmitate curve peaked at 2 hours after low fibre muffins and 3 hours after high fibre muffins. This may indicate that the TG curve peaked earlier than 2 hours after low fibre muffins. Unfortunately, assessments were not made before 2 hours of the postprandial period. Delayed entry of chylomicrons into the circulation can be caused by several mechanisms, such as delayed gastro-intestinal transit time. However, insoluble fibres have little effect on gastric emptying and small bowel transit and accelerate colonic transit (Spiller, 1994). A decreased digestion of dietary fat in the small intestine through reduction of pancreatic lipase (Mann & Skeaff, 1998) could also be responsible. It has been shown *in vitro* that wheat bran can decrease pancreatic lipase activity (Lairon *et al.* 1985). Thirdly, decreased gastro-intestinal hormone (gastrin, secretin, cholecystokinin and

gastric inhibitory peptide {GIP}) secretion may decrease insulin secretion (Meyer, 1988). Very few studies have examined the effect of dietary fibre, especially wheat bran or insoluble fibre, on gastro-intestinal hormone secretion. Morgan *et al.* (1993) demonstrated no effect of wheat bran on GIP levels, while GIP values were lower after type two diabetic patients consumed a high fibre breakfasts (Hagander *et al.* 1984). Wheat bran has also been shown to increase secretin levels in pigs (Langlois *et al.* 1987).

Because the serum TG and plasma retinyl palmitate values did not differ after 4 hours, one may argue that the increased serum TG levels after the high fibre muffins were only transient, that the overall clearance of dietary fat between test meals did not differ and thus, might not be detrimental. However, this initial increased serum TG levels probably caused the significantly lower HDL-C levels after high fibre muffins compared to low fibre muffins. In a study by Anderson *et al.* (1995) HDL-C levels also decreased significantly after both low fibre and high fibre (26.1g total fibre, 13.2g soluble fibre) diets, although the two diets did not differ significantly. The decrease in HDL-C can be explained by the remodelling of lipids in lipoprotein particles. An increased presence of chylomicrons in the plasma due to delayed clearance, results in increased transfer of TG from chylomicrons into HDL_2 in exchange for CE from HDL through lipid transfer proteins. The TG enriched HDL_2 are converted to HDL_3 by HL, resulting in decreased HDL_2 levels. The metabolism and uptake of HDL is thus increased so that the levels of these so-called anti-atherogenic lipoproteins are decreased (O'Meara *et al.* 1992; Patsch *et al.* 1987). It is unclear what the extent of this postprandially lowered HDL-C levels on the risk for CHD will be. However, if one consumes high fibre products at every meal, the HDL-C levels might be decreased for long periods, since most of the time is usually spent in the postprandial state. This postprandial higher TG, lower HDL-C responses after a high fibre test meal may also be in accordance with studies that examined the chronic intake of high carbohydrate, low fat diets that resulted in lowered fasting HDL-C (Katan *et al.* 1995) and increased TG levels (West *et al.* 1990).

As shown in the literature (Anderson *et al.* 1995; Cara *et al.* 1992) TC levels decreased significantly from baseline with both test meals. Although not statistically significant, the high fibre muffins tended to reduce the TC response curve more than the low fibre muffins. This observation is in accordance with results from Cara *et al.* (1992). Decreased postprandial cholesterol levels after meal intake may be explained by reduced endogenous synthesis of

cholesterol when intestinal dietary cholesterol enters the circulation (Brown & Goldstein, 1983).

Plasma $FVII_c$ was not significantly altered from baseline for either of the two test meals even though serum TG levels increased significantly. A possible explanation might be that the fat content of the test meals (30.5g) were too low to activate factor VII. Most of the studies in the literature that reported positive associations between fat intake and $FVII_c$ used very large amounts of fat, for example 1g fat/1 kg body weight (Freese & Mutanen, 1995; Salomaa *et al.* 1993) or 70-120g fat (Larsen *et al.* 1997; Oakley *et al.* 1998; Sanders *et al.* 1996). Test meals that provided less than 70g of fat did not have an effect on $FVII_c$ (Roche & Gibney, 1997). If, in a diet of 12 000 kJ for moderately active men (Mahan & Escott-Stump, 1996), 30% of total energy is provided by fat, it is equal to 95g of fat/day. If three meals are eaten with equal fat contents, 32g of fat is provided per meal. Thus, 30.5g fat can be regarded as a physiological amount.

In conclusion, high fibre muffins caused more gradual serum TG and retinyl palmitate response curves compared to low fibre muffins resulting in higher serum TG and plasma retinyl palmitate values at 3 hours. This higher serum TG en plasma retinyl palmitate values after high fibre muffins caused significantly lower HDL-C levels compared to low fibre muffins. Before final conclusions regarding this possible detrimental effect of wheat bran is made, these findings need to be confirmed by other postprandial studies. The possible mechanisms responsible for these findings need to be further examined. The effects of wheat bran on intestinal digestion and absorption of dietary fat, activity of digestive enzymes, secretion of gastro-intestinal hormones and chylomicron catabolism should receive attention in such studies.

In this study, with the intake of normal physiological amounts of fat, incorporated into a baked product, serum TG levels increased but did not activate factor VII.

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CHAPTER 7

EXTRUDED DRY BEANS AND SERUM LIPOPROTEIN AND PLASMA HAEMOSTATIC FACTORS IN HYPERLIPIDAEMIC MEN

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Abstract

Objective: To examine the effects of the inclusion of extruded dry beans in the diet on serum lipoprotein, plasma fibrinogen, plasma viscosity and plasminogen activator inhibitor 1 (PAI-1) levels.

Subjects and study design: Twenty-two free living hyperlipidaemic men participated in this randomised, controlled, cross-over study. The subjects were randomly assigned to one of two groups. After a run-in period of 4 weeks, during which subjects followed their normal diet with the exclusion of dry beans, group A had to include 110g/day of extruded dry beans in the form of baked products, for 4 weeks while group B continued with the run-in diet. A washout period of 4 weeks followed after which the experimental intervention was crossed-over. Anthropometric measurements, serum lipoproteins and haemostatic variables were measured with standard methods and dietary intakes were estimated with five-day dietary records at the beginning and end of each experimental period.

Results: Compliance was determined as 83.5% with a mean intake of 91.9g/day extruded dry beans. Extruded dry beans did not have significant effects on total serum cholesterol, low density lipoprotein cholesterol, triglycerides, apoprotein A or B, plasma fibrinogen and plasma viscosity concentrations. High density lipoprotein cholesterol concentrations decreased in both the dry bean and control periods. Lipoprotein (a) concentrations increased with intake of extruded dry beans, but this increase was probably not due to an independent effect of extruded dry beans. PAI-1 levels were significantly lower after the intake of extruded dry beans compared to the control period.

Conclusion: The inclusion of 91.9g extruded dry beans per day in the diet had no effects on serum lipoproteins, plasma fibrinogen and viscosity levels but decreased PAI-1 levels.

Descriptors: extrusion, dry beans, lipoproteins, fibrinogen, viscosity, plasminogen activator inhibitor 1, PAI-1

Sponsorship: Dry bean Producers Organisation (South Africa) and the Potchefstroom University for Christian Higher Education, Potchefstroom, South Africa

Introduction

Cerebrovascular and coronary heart disease (CHD) are of the most important causes of mortality and morbidity among South Africans (Bradshaw *et al,* 1995) and other Western societies (Murray & Lopez, 1996). It has been shown that increased lipoprotein (Shrapnel *et al,* 1992) and plasma fibrinogen (Danesh *et al,* 1998; Wilhelmsen *et al,* 1984) as well as decreased fibrinolytic activity (Meade *et al,* 1993; Meade *et al,* 1996) are independent risk factors for these diseases. Increased plasma plasminogen activator inhibitor 1 (PAI-1) level decreases fibrinolytic activity (Aznar *et al,* 1988). It has also been shown that increased levels of PAI-1 are associated with increased risk for CHD (Scarabin *et al,* 1998). It is possible that these risk factors can be manipulated by dietary interventions (Shrapnel *et al,* 1992; Vorster *et al,* 1997).

Dry beans are rich and economical dietary sources of plant protein, complex carbohydrates, soluble and insoluble dietary fibre components and a variety of minerals and vitamins. Dry beans have a low energy, fat and sodium content. This nutrient composition meets the requirements of the prudent diet, recommended for the prevention and treatment of chronic degenerative Western diseases (Vorster & Venter, 1994). In recent years extrusion cooking has become one of the most popular new processes developed by the food and feed industry (Gujska & Khan, 1990). Extrusion of dry beans is a relatively new process and provides an economically and convenient alternative to cooking and canning and provides a variety of options for ingesting this health food. Extrusion can be described as a high temperature (125°C), low liquid (<25%), short time (<60sec) processing technique (Harper, 1995).

Although the effects of extruded dry beans on lipoprotein levels have not yet been examined, a few studies have reported on the effects of dry beans (cooked or canned), with inconsistent results. In the studies of Cobiac *et al* (1990) and Mackay & Ball (1992) dry beans had no effects on total cholesterol (TC), triglycerides (TG) and low density lipoprotein cholesterol (LDL-C). Anderson *et al* (Anderson *et al,* 1984; Anderson *et al,* 1990) showed in metabolic ward studies independent decreases in lipoprotein concentrations with intake of dry beans. Shutler *et al* (1989) also reported decreased lipoprotein concentrations when diets high in dry beans were consumed. However, these decreases were due to decreased fat intakes caused by a replacement effect in the dry bean diet.

The effects of dry beans on haemostatic variables have not been studied. But, dry beans are a rich source of dietary fibre, especially soluble fibre (Vorster & Venter 1994), that may have beneficial effects on haemostatic factors (Koepp & Hegewisch, 1981; Landin *et al,* 1992; Sundell & Rånby, 1993; Venter *et al,* 1990; Venter *et al,* 1991).

The objectives of this study were to examine the effects of the inclusion of extruded dry beans in the diet on serum lipoprotein, plasma fibrinogen, plasma viscosity and PAI-1 levels.

Methods

Subjects

The study was approved by the Ethics Committee of the Potchefstroom University for Christian Higher Education (Ethics no. HHK3M3-92). An informed consent form was signed by each of the 22 hyperlipidaemic men who regularly attended the Lipid Clinic at the Potchefstroom University and who voluntarily participated in this study. Their median [quartile range] age was 48.0 [7.0] years.

The exclusion criteria for selection of subjects were: smoking, body mass index (BMI) >30kg/m^2, baseline fasting blood glucose >5.55mmol/L, baseline serum TC levels <5.2mmol/L, baseline serum TG levels >4mmol/L, familial hypercholesterolaemia (FH) and the use of lipid lowering drugs. FH was assessed with a polymerase chain reaction based method that detects LDL-receptor mutations on exon 4 or 9 of the LDL-receptor gene (Kotze *et al,* 1994) or by elevated LDL-C levels (above 7.5mmol/L) combined with tendon xanthomata and a known family history of CHD.

Study design

This study was done under free living conditions using a randomised, controlled, cross-over study design. The subjects were randomly assigned to one of two groups, namely group A (n=11) or group B (n=11). During a run-in period of 4 weeks the subjects followed their normal diet with the exclusion of dry beans. After the run-in period, group A had to include 110g/day of extruded dry beans, in the form of baked products, into their diet while group B continued to follow the run-in diet. After a wash-out period of 4 weeks where all the subjects

had to follow the run-in diet, the experimental intervention was crossed-over. The subjects had to, as in the studies of Jenkins *et al* (1983) and Mackay & Ball (1992), exchange carbohydrate foods for the extruded dry bean products. To assist them in doing so, they were provided with a carbohydrate food exchange list with directions and examples of how to use it. The 110g extruded dry beans provided 1156 kJ (276 kcal), 0.99g fat, 41.3g carbohydrate, 24.5g protein (as analysed by the laboratory of Senwes Bpk., Viljoenskroon, South Africa) and 15g total non-starch polysacharides (NSP), 9.2g insoluble NSP and 5.7g soluble NSP (as analysed with the Englyst Fiberzym® Kit, Dunn Nutrition Centre, Cambridge, UK, Cat.no. 7367722).

Preceding the study all the subjects and their wives were invited to an information session where all aspects of the study were discussed and the necessary instructions and directions given. To ensure compliance to the experimental intervention, the subjects could taste some of the extruded dry bean products during this session. The acceptability of the extruded dry bean products were found to be high. The subjects were also asked to maintain their habitual diet, alcohol consumption and physical activity for the duration of the study.

Fasting blood samples were drawn and anthropometric measurements (height, weight, waist and hip circumferences) were taken at the beginning and end of each experimental period. Two baseline blood samples, one week apart, were drawn and the means of the two calculated. BMI (kg/m^2) was calculated.

Extruded dry bean baked products

The recipes for the extruded dry bean baked products were developed by the Dry bean Producers Organisation (South Africa) and included corn savoury bread, chocolate cake, banana bread and raisin muffins.

Dietary intakes

Dietary intakes were estimated with five day dietary records at the beginning and end of each experimental period. The dietary records were also used to determine compliance to the dietary intervention. The five consecutive days on which records were kept were randomly determined and included 1 weekend day. The Medical Research Council's food quantities (Langenhoven *et al,* 1991a) and food composition (Langenhoven *et al,* 1991b) tables were

used to encode food items, to translate household measures to grams and to analyse nutrient intakes.

Blood samples

The subjects were required to fast overnight (12 hours). Venous blood samples were collected using a 21-gauged scalp infusion set. All samples were drawn with minimal stasis between 07:00 and 10:00 to avoid effects of diurnal variation. EDTA blood was used for the analysis of haematocrit and haemoglobin. Clotted, citrated and EDTA blood were centrifuged for 15 minutes at 2000*g* to yield serum for lipoproteins, plasma for haemostatic variables and plasma for viscosity measurements, respectively. Fresh EDTA plasma was used for viscosity measurements and the citrated plasma and serum were divided into aliquots and frozen at 72°C within 30 minutes for later analysis.

Experimental methods

TC concentrations were determined enzymatically with the Technicon Omnipak-method (SM-4-0139/86, Bayer, Puteaux Cedex). LDL-C was determined with the CHOD-Iodide method (Merck, Darmstadt, Germany). LDL-C was precipitated by heparin at its iso-electric point (pH 5.12). After centrifugation, the high density lipoprotein cholesterol (HDL-C) and the very low density lipoprotein cholesterol remained in the supernatant and were determined with an enzymatic method. The LDL-C concentration was calculated according to the following formula: LDL-C=TC – cholesterol in supernatant. Apoprotein (apo) A and apoB were determined with an immuno-chemical method (Behring Institute, Marburg, West Germany, antiserum for human apoA {code no. OUED}, and human apoB {code no. OSAN}). Lipoprotein (a) (Lp(a)) was determined with a radio-immunological method (Pharmacia Apo(a) RIA). Plasma fibrinogen was measured with the Clauss method using an ACL-200 automated coagulation analyser and reagents from Instrumentation Laboratories (IL) (Milan, Italy) (Coefficient of variance {CV} = 2.38%). Fibrinogen standards and controls were purchased from IL and from the National Institute for Biological Standards and Control (code 89/644, NIBSC, Hertfordshire, UK). PAI-1 was measured with an indirect enzymatic method (Spectrolyse pL, Biopool, Umeå, Sweden, Cat.no. 101201) (intra CV = 10.37%; inter CV = 3.63%). Plasma viscosity was measured with a Brookfield Digital Viscometer (Model DV-1+ Version 3.0, Stoughton, USA) (intra CV = 3.13%; inter CV = 3.48%). Haematocrit was

determined with the capillary tube method and haemoglobin with a calorimetric method (Boehringer Mannheim, Cat.no. 124729).

Statistical analysis

The computer software package Statistica® was used for these analyses. Because a normal distribution of data could not be assumed, significant differences within and between groups were determined with the Wilcoxon matched pairs test. All data are presented as median [quartile range]. A *P*-value of less or equal to 0.05 was regarded as being statistically significant.

Results

The median [quartile range] levels of nutrient intakes and experimental variables during the study are summarised in Tables 1-5 and the mean food intakes per day of specific foods are illustrated in Figure 1.

Anthropometric measurements did not change during the study (Table 1). Haematocrit levels were also not effected, but haemoglobin levels tended to decrease with intake of extruded dry beans and decreased significantly in the control group (Table 1). These small decreases were probably not of any clinical significance, because reference values for haemoglobin in men are 8.68-11.16 mmol/L (Lee & Nieman, 1993).

According to Figure 1, compliance to the experimental intervention was relatively good. The mean intake of extruded dry beans per day was 91.9g which is 83.5% of the 110g/day that had to be consumed. The acceptability of the extruded dry bean products were good and reported side effects included a feeling of prolonged satiety and flatulence, which diminished as the study progressed. As shown in Figure 1, the subjects exchanged, as was instructed, carbohydrate rich foods, mainly bread and breakfast cereals, for the extruded dry bean products.

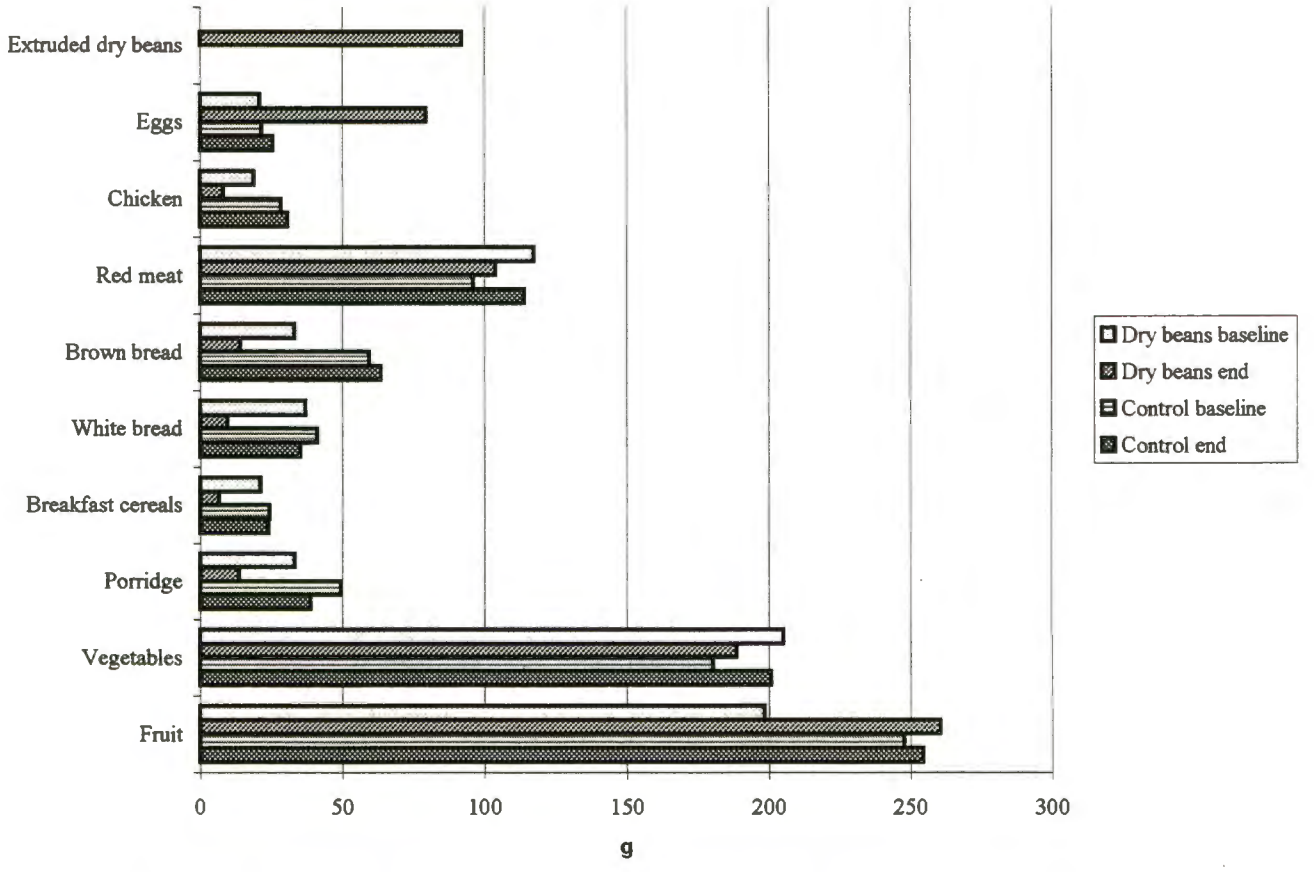

Figure 1 Mean changes in food intake per day of specific foods during the study.

Table 1 Median [quartile range] changes in anthropometric, haematocrit and haemoglobin levels during the study

	Extruded dry bean period (n=22)				Control period (n=22)			
	Baseline		End		Baseline		End	
	Median	QR	Median	QR	Median	QR	Median	QR
Weight (kg)	86.5	10.2	85.5	9.2	86.9	9.2	86.4	9.8
BMI (kg/m^2)	27.9	3.5	27.6	3.3	27.3	3.4	27.1	3.3
Waist (cm)	96.3	9.0	95.5	10.0	95.5	9.5	96.0	10.0
Hip (cm)	102.0	5.0	102.0	6.0	102.0	6.0	102.0	6.0
Waist/hip ratio	0.93	0.05	0.94	0.05	0.93	0.05	0.93	0.06
Haematocrit (%)	45.0	5.0	45.0	4.0	45.0	3.8	45.0	4.0
Haemoglobin (mmol/L)	11.0	0.7	10.5	1.0	11.0*	1.6	10.1*	0.9

QR: Quartile range; BMI: Body mass index
Medians [quartile ranges] with the same symbol differed significantly ($p \leq 0.05$).

Table 2 Median [quartile range] changes in energy and macronutrient intakes and energy distribution during the study

		Extruded dry bean period (n=22)				Control period (n=22)			
		Baseline		End		Baseline		End	
		Median	QR	Median	QR	Median	QR	Median	QR
Energy	kJ (kcal)	8679 (2074)	2485	9047 (2162)	1663	8512 (2034)	3220	8261 (1974)	3513
Protein	g	90.0	23.6	94.4$^{\#}$	27.2	81.9	18.0	78.8$^{\#}$	30.6
	%E	16.0*	1.9	17.0*	2.6	15.6	3.2	16.5	5.1
Plant protein	g	23.0*	7.0	34.3$^{*\#}$	11.4	22.2	6.1	24.2$^{\#}$	11.4
	%E	4.5*	0.9	5.8$^{*\#}$	2.2	3.9	1.2	3.8$^{\#}$	1.7
Animal protein	g	64.8	17.3	56.1	20.8	58.7	18.1	57.3	31.8
	%E	11.3	2.5	10.4	5.6	9.8	6.4	10.7	4.6
Fat	g	87.2	28.1	74.6	21.3	73.8	33.1	77.8	38.7
	%E	35.7*	8.2	33.0*	4.1	32.8	7.1	35.1	7.1
SFA	g	30.6*	12.8	22.4*	7.0	27.1	10.3	26.9	11.5
	%E	12.3*	2.0	9.4$^{*\#}$	1.6	12.4	2.2	11.6$^{\#}$	1.8
MUFA	g	31.2	9.7	31.3$^{\#}$	9.7	25.8	15.3	27.7$^{\#}$	11.4
	%E	12.5*	2.9	13.7*	1.5	12.5	3.5	12.6	3.5
PUFA	g	18.2$^{\#}$	8.7	16.9	5.9	15.4$^{\#}$	10.7	15.8	10.7
	%E	7.5$^{\#}$	2.0	7.2	1.3	6.6$^{\#}$	2.1	7.4	3.7
Cholesterol	mg	342*	119	470$^{*\#}$	162	297	101	337$^{\#}$	198
Carbohydrate	g	227.6	60.4	251.5$^{\#}$	70.9	228	63.5	184.1$^{\#}$	96.5
	%E	46.4*	11.7	50.6*	8.2	48.8	9.7	50.1	11.0
Dietary fibre	g	19.5*	9.6	24.6$^{*\#}$	11.1	17.5	8.9	19.5$^{\#}$	10.1
Alkohol	g	9.3	21.5	7.3	13.1	8.5	12.4	6.8	16.8

QR: Quartile range; %E: Percentage from total energy intake; SFA: Saturated fatty acids; MUFA: Mono-unsaturated fatty acids; PUFA: Polyunsaturated fatty acids
Medians [quartile ranges] with the same symbol differed significantly ($p \leq 0.05$).

As shown in Table 2, total energy intake did not change during the study. None of the nutrient intakes changed during the control period (Tables 2 and 3). The inclusion of extruded dry beans in the diet caused some expected changes in nutrient intake. Protein intake increased significantly due to the significant increased intake of plant protein of which dry beans is a rich source (Vorster & Venter, 1994). Animal protein intake tended to decrease (not significantly). Percentage energy from fat decreased significantly mainly because of a decrease in saturated fatty acid (SFA) intake. The small decrease in meat (Figure 1) and animal protein intake may be responsible for this decrease in SFA intake. The high grade of satiation caused by the extruded dry bean products probably resulted in the lower intake of meat. A small, but significant increase in mono-unsaturated fatty acid (MUFA) intake occurred probably because

of canola oil (rich source of MUFA) that was used in the extruded dry bean products. The median cholesterol intake level increased significantly due to the use of eggs (Figure 1), that played an important role in the acceptability of the extruded dry bean products. Median carbohydrate intake was also slightly increased. As expected, dietary fibre increased significantly due to the inclusion of extruded dry beans in the diet. The subjects did not change their alcohol intake during the study. Extruded dry bean intake caused significantly increased intakes of iron, magnesium, potassium, copper, thiamine and folate (Table 3). The significant increase in vitamin D intake can be attributed to the eggs used in the extruded dry bean products. Because the vitamin E content of the canola oil used in the extruded dry bean products was unknown and thus not included in the nutrient analyses, vitamin E levels appear to be decreased.

Table 3 Median [quartile range] changes in micronutrient intakes during the study

		Extruded dry bean period (n=22)				Control period (n=22)				RDA
		Baseline		End		Baseline		End		
		Median	QR	Median	QR	Median	QR	Median	QR	
Calcium	mg	637	239	746	325	642	392	670	278	800
Iron	mg	12.0*	4.1	16.0$^{*\#}$	3.1	12.3	5.5	12.1$^{\#}$	3.2	10.0
Magnesium	mg	313*	55	428$^{*\#}$	84	304	93	316$^{\#}$	96	350
Phosphorus	mg	1318	285	1550$^{\#}$	354	1284	460	1220$^{\#}$	496	800
Potassium	mg	2891*	631	3881$^{*\#}$	974	2726	792	2869$^{\#}$	760	2000
Sodium	mg	2187	895	2160	959	2319	1100	2097	733	500
Zinc	mg	11.8	2.6	12.1	2.3	10.6	3.6	12.1	3.1	15.0
Copper	mg	1.4*	0.3	1.9$^{*\#}$	0.69	1.3	0.5	1.4$^{\#}$	0.6	1.5
Thiamine	mg	1.22*	0.50	1.56$^{*\#}$	0.65	1.21	0.44	1.14$^{\#}$	0.52	1.50
Riboflavin	mg	1.63	0.50	1.90	0.56	1.58	0.65	1.51	0.92	1.70
Vitamin B_6	mg	1.82	0.53	1.85	0.69	1.55	0.83	1.69	0.85	2.00
Vitamin B_{12}	µg	5.5	3.0	5.7	2.4	4.3	1.7	5.7	4.2	2.0
Folate	µg	210*	76	546$^{*\#}$	170	251	71	252$^{\#}$	128	200
Nicotinic acid	mg	21.8	8.7	19.9	5.5	21.6	10.5	21.7	9.6	19.0
Vitamin C	mg	75	52	78.5	92	67	124	80	100	60
Vitamin A	RE	760	966	945	880	1117	940	1153	784	1000
Vitamin D	µg	4.7*	2.5	6.9$^{*\#}$	3.5	4.3	4.3	4.7$^{\#}$	4.5	5.0
Vitamin E	mg	16.7*	9.1	10.1*	6.2	10.3	6.6	14.0	8.3	10.0

RDA: Recommended Dietary Allowances (Food and Nutrition Board, 1989); QR: Quartile range
Medians [quartile ranges] with the same symbol differed significantly ($p \leq 0.05$).

As shown in Table 4, extruded dry bean intake had no effects on TC, TG, LDL-C, apoA or apoB levels. HDL-C levels decreased significantly during both the extruded dry bean and control periods. Lp(a) levels increased significantly with the intake of extruded dry beans. As can be deducted from the high interquartile range levels, great individual variations occurred, where Lp(a) values ranged from <30 U/L to >1500 U/L. For better interpretation, the changes in individual values were categorised according to risk levels (Table 4). The number of subjects in the high risk category (>500 U/L) increased by 2 in both the extruded dry bean and control periods. Thus, the increase in Lp(a) levels with extruded dry beans was probably not due to an independent effect of extruded dry beans.

Plasma fibrinogen and viscosity levels did not change during the study (Table 5). Median plasma PAI-1 level at the end of the extruded dry bean period was significantly lower compared to the control period.

Table 4 Median [quartile range] changes in serum lipoprotein levels during the study

	Extruded dry bean period (n=22)				Control period (n=22)			
	Baseline		End		Baseline		End	
	Median	QR	Median	QR	Median	QR	Median	QR
TC (mmol/L)	6.07	0.85	6.15	0.70	6.18	0.60	6.15	1.00
TG (mmol/L)	1.64	1.65	1.75	1.31	1.74	1.24	1.58	1.20
HDL-C (mmol/L)	0.97*	0.16	0.84*	0.25	0.96*	0.25	0.89*	0.31
LDL-C (mmol/L)	4.71	0.91	5.09	0.64	4.86	0.60	4.87	0.86
ApoA (g/L)	1.30	0.13	1.36	0.25	1.35	0.15	1.41	0.24
ApoB (g/L)	1.35	0.20	1.37	0.22	1.37	0.33	1.36	0.32
Lp(a) (U/L):	230.0*	325.0	279.9*#	379.4	251.2	365.0	259.2#	465.6
0-300 U/L (n)	13		12		11		12	
300-500 U/L (n)	7		6		7		4	
>500 U/L (n)	2		4		4		6	

QR: Quartile range; n: Number of subjects; TC: Total cholesterol; TG: Triglycerides; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; apo: Apoprotein; Lp(a): Lipoprotein (a)
Medians [quartile ranges] with the same symbol differed significantly ($p \leq 0.05$).

Table 5 Median [quartile range] changes in plasma fibrinogen, plasminogen activator inhibitor 1 (PAI-1) and viscosity levels during the study

	Extruded dry bean period (n=22)				Control period (n=22)			
	Baseline		End		Baseline		End	
	Median	QR	Median	QR	Median	QR	Median	QR
Fibrinogen (g/L)	2.97	0.50	3.14	0.80	3.12	0.36	3.03	0.59
PAI-1 (U/mL)	12.40	6.48	11.13[#]	7.73	11.20	9.24	13.81[#]	11.13
Viscosity (cP)	1.29	0.14	1.31	0.13	1.32	0.11	1.31	0.07

QR: Quartile range
Medians [quartile ranges] with the same symbol differed significantly ($p \leq 0.05$).

Discussion

The salient observations of this study on free living hyperlipidaemic men were that extruded dry beans had no effect on serum lipoprotein, plasma fibrinogen or viscosity levels, but that it decreased plasma PAI-1 levels.

Although the effects of extruded dry beans on lipoprotein levels have not yet been examined, a few studies have reported on the effects of dry beans (cooked or canned), with inconsistent results. In the studies of Cobiac *et al* (1990) and Mackay & Ball (1992) dry beans had no effects on TC, TG and LDL-C. The studies done by Anderson *et al* (Anderson *et al,* 1984; Anderson *et al,* 1990) in metabolic wards showed independent decreases in lipoprotein concentrations with intake of dry beans. Shutler *et al* (1989) also reported decreased lipoprotein concentrations with intake of dry beans, but these decreases were probably due to decreased fat intake caused by the dry bean diet.

In this study dietary changes that occurred because of the inclusion of extruded dry bean products in the diet complicates interpretation and explanation of the lack of effect on lipoprotein levels. The dietary changes, other than those caused by dry beans itself (increased dietary fibre and plant protein intakes), that could have effects on lipoprotein levels were firstly, increased dietary cholesterol intakes due to the eggs used in the extruded dry bean products. Grundy *et al* (1988) showed that an increase of 100mg per 4184 kJ/day (1000kcal/day) dietary cholesterol could increase serum cholesterol levels with 0.26 mmol/L.

SFA intakes, that decreased in this study, could also have effected the lipoprotein levels. Decreased SFA intake lowers TC and LDL-C concentrations (Morgan *et al,* 1993). Thirdly, the increased MUFA intake in this study would probably not have affected the lipoprotein levels independently, since its effect regarding serum lipoproteins is neutral (Hegsted *et al,* 1993; Mensink & Katan, 1992). The interpretation of the data could probably be simplified if the effects on serum lipoproteins due to these dietary changes could be predicted. Howell *et al* (1997) composed such prediction equations from a meta-analysis of the relevant literature. ΔTC (mg/dL) = 1.918 × ΔSFA(% of total energy intake (%E)) - 0.900 × Δpolyunsaturated fatty acid (PUFA)(%E) + 0.0222 × Δdietary cholesterol(mg); ΔLDL (mg/dL) = 0.287 × ΔSFA(%E) + 0.129 × Δtotal fat (%E); ΔTG (mg/dL) = 0.0144 × Δdietary cholesterol(mg) - 1.066 × ΔPUFA(%E) - 0.919 × Δtotal fat (%E). If the data from the present study is used in these equations, no changes in either TC, LDL-C or TG could be expected.

The high content of soluble fibre in dry beans seems to be the main factor in the cholesterol lowering effect of dry beans (Anderson & Gustafson, 1987; Geil & Anderson, 1994; Uebersax *et al,* 1991). The significant increase in dietary fibre caused by the inclusion of extruded dry beans in this study was probably not sufficient to cause a hypocholesterolaemic effect. The amount of total and soluble NSP provided by the 91.9g extruded dry beans (12.5g total NSP and 4.8g soluble NSP) were analysed but unfortunately, because the South African food composition tables only includes total dietary fibre intake, the amount of soluble fibre provided by the diet could not be determined. Since the mean intake of fruit and vegetables, good sources of soluble fibre (Englyst *et al,* 1988), remained relatively unchanged and the greatest fraction of insoluble fibre from extruded dry beans probably replaced the insoluble fibre rich carbohydrate foods, the increased median dietary fibre intake of 5.1g/day was probably mainly from soluble fibre provided by extruded dry beans. The amount of soluble fibre provided by dry beans in the two metabolic ward studies (Anderson *et al,* 1984; Anderson *et al,* 1990) that reported cholesterol lowering effects were 10 and 4g respectively. The studies of Cobiac *et al* (1990) and Mackay & Ball (1992) that did not report cholesterol lowering effects provided extra amounts of 7g soluble and 5g total dietary fibre. Thus, the intake of 4g of soluble fibre from dry beans is probably sufficient to lower cholesterol concentrations under metabolic ward conditions, but not under free living conditions.

The possibility that the extrusion process could have caused chemical and structural changes of the dry beans and that this might be the reason for the lack of effects on serum lipoprotein levels, can not be ignored. However, the nutrient composition of the dry beans before and after extrusion were compared and did not differ with regard to total energy, fat, carbohydrate, protein, total, soluble and insoluble NSP content. Lintas & Cappelloni (1992) showed that the percentage resistant starch in cooked compared to extruded white beans did not differ.

In the literature the effects of dry beans on HDL-C levels are controversial. Some studies reported lowering effects (Anderson *et al,* 1990; Shutler *et al,* 1989), others increasing effects (Mackay & Ball, 1992) while some reported no effects (Anderson *et al,* 1984; Cobiac *et al,* 1990; Jenkins *et al,* 1983). The decrease in serum HDL-C concentrations in this study were probably not an independent effect of extruded dry beans because a lowering effect was also seen in the control group. Very low fat diets can decrease HDL-C levels (Howell *et al,* 1997; Mensink & Katan, 1992). Although the total and SFA intake decreased with the intake of extruded dry beans, this change was not seen in the control period, making this mechanism inapplicable to this study. Large intra-individual variations in HDL-C levels have been noticed in the Lipid Clinic at the Potchefstroom University for Christian Higher Education (Vorster *et al,* 1994). The decreases in HDL-C might, thus be due to chance and be explained by subject variations.

In this study Lp(a) concentrations increased with the intake of extruded dry beans. As explained in the results section, this increase was probably not due to an independent effect of extruded dry beans. The effect of dry beans on Lp(a) have not yet been reported in the literature. It is known that Lp(a) is hardly effected by dietary intervention (Harris, 1997).

Data on the effects of dietary factors on fibrinogen, viscosity and PAI-1 levels are very limited and the effects of dry beans on these variables have not yet been examined. The independent effects of fat and cholesterol intake on plasma fibrinogen is unknown, but Vorster *et al* (1987) have reported increased fibrinogen levels in subjects who followed a diet high in fat, cholesterol and animal protein and low in fibre compared to a control group. The effect of fibre intake on plasma fibrinogen levels seems to be controversial. Plasma fibrinogen levels decreased with the intake of soluble fibre in diabetic children (Koepp & Hegewisch 1981) and in animals (Venter *et al,* 1990; Venter *et al,* 1991), while Landin *et al,* (1992) showed no effect in humans. In the studies of Marckman *et al* (1993) and Djoussé *et al* (1998) low fat,

high fibre diets did not effect fibrinogen levels in humans. Possible reasons for the lack of effect on fibrinogen levels in the present study might be that the increase in fibre intake was not sufficient to lower fibrinogen levels. Another reason might be that fibrinogen concentrations were in the normal range making it difficult to lower it further. Thirdly, other variables that may affect fibrinogen levels namely serum TC (Lee *et al,* 1990) and LDL-C (Møller & Kristensen, 1991) were unchanged during the study. Plasma viscosity levels did not change during the study. This may be explained by the lack of effect on plasma fibrinogen which is an important determinant for plasma viscosity (Lowe, 1986). The median plasma PAI-1 level was significantly lower after the intake of extruded dry beans compared to the control period. Although it has been shown that PAI-1 can decrease with lowering fat intakes (Sundell *et al,* 1991) this lowering effect has not been seen with modest fat reductions (Marckmann *et al,* 1992; Velthuis-te-Wierik *et al,* 1996) as was the case in this study. The lowering of PAI-1 levels might thus be ascribed to the significant increase in fibre intake. Djoussé *et al* (1998) reported a negative association between dietary fibre intake and PAI-1. Soluble fibre rich products decreased plasma PAI-1 levels (Landin *et al,* 1992; Sundell & Rånby, 1993).

In conclusion, the intake of 91.9g/day extruded dry beans had no effects on serum lipoproteins, plasma fibrinogen and viscosity levels but plasma PAI-1 levels different significantly after extruded dry bean intake compared to the control period. Thus, the inclusion of extruded dry beans in the diet may increase the fibrinolytic potential (Aznar *et al,* 1988) and thereby contribute to decrease CHD risk (Meade *et al,* 1993; Meade *et al,* 1996).

Even though extruded dry beans did not have the desired hypocholesterolaemic effects, dry beans, because it is a rich and economical source of plant protein, complex carbohydrates, soluble and insoluble dietary fibre components and a variety of minerals and vitamins and it is low in energy, fat and sodium content (Vorster & Venter, 1994), forms an important part of the hypocholesterolaemic diet. Processing techniques to develop alternative and convenient ways in which this health food can be consumed are encouraged but care should be taken with the development of the extruded dry bean products so that acceptable products that fits into the hypocholesterolaemic diet are developed.

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CHAPTER 8

GENERAL SUMMARY, RECOMMENDATIONS, DISCUSSION AND CONCLUSIONS

1. INTRODUCTION

In this chapter, a summary of the main findings from the five studies reported in this thesis will be given. Recommendations to Government, the food industry and health professionals will be made from these findings. The results from each study are discussed, interpreted, elucidated and compared to the relevant literature in the separate chapters. The general discussion in this chapter will therefore focus on the compatibility of the main findings and recommendations following from each study with the consensus dietary guidelines for the management of hyperlipidaemias in South Africa.

2. SUMMARY OF MAIN FINDINGS

The salient observations of the five studies reported in this thesis were:

2.1 Alcohol

- The VIGHOR study showed that the reported consumption of alcohol by white South Africans from the industrialised communities of Witbank and Vanderbiljpark was low compared to findings in the international literature.

- Alcohol consumption was positively associated with high-density lipoprotein cholesterol (HDL-C) in both sexes. However, this protective effect was partly counter-balanced by a positive association between alcohol consumption and blood pressure in men.

- No association of alcohol consumption with total serum cholesterol, low density lipoprotein cholesterol or triglyceride (TG) concentrations were shown.

2.2 Sugar

- In the VIGHOR population added sugar intake was not associated with body mass index.

- Added sugar intake was negatively associated with fat intake.

- Sugar intake did not constitute a major risk to nutritional inadequacy in men. The associations between added sugar intake and most of the micronutrients took on inverse U-shaped curves. Except for vitamin D, the micronutrient intakes were >67% of the recommended dietary allowance, irrespective of sugar intake. Micronutrient intakes were highest in men with mean added sugar intakes of 16.4% of total energy (%E) (117g/day) (15-24 year) and 15.2%E (96g/day) (25-64 year).

- In women, high added sugar intake contributed to their already micronutrient-inadequate diets. They were, probably because of their low energy intakes, more at risk of nutrient inadequacy with increased sugar intake, especially with regard to fibre, iron, magnesium, copper, thiamine and vitamin B_6. Micronutrient intakes were highest in women with added sugar intakes of 7.1-17%E (33-82g/day) (15-24 year) and 3.4-13.3%E (12-50g/day) (25-64 year).

2.3 Lecithin

- The intake of 20g/day soya bean lecithin had no independent effects on serum lipoprotein, plasma fibrinogen or macro molecular protein complex (MPC) levels in free living hyperlipidaemic men in a double-blind study which controlled for possible indirect effects.

- Sunflower oil treatment resulted in reduced MPC levels.

2.4 High fibre *vs* low fibre meals on postprandial responses

- Although the overall responses of serum TG, plasma retinyl palmitate and serum HDL-C did not differ between the two test meals, high fibre muffins caused more gradual serum TG and plasma retinyl palmitate response curves than low fibre muffins, resulting in higher serum TG and plasma retinyl palmitate and lower serum HDL-C levels at 3 hours after the test meal.

- Factor VII coagulant activity was not significantly altered from baseline for either of the two test meals, even though serum TG levels increased significantly.

2.5 Extruded dry beans

- The intake of 91.9g/day extruded dry beans had no effects on serum lipoprotein, plasma fibrinogen or viscosity levels but decreased plasminogen activator inhibitor 1 (PAI-1) levels in free living hyperlipidaemic men in a randomised controlled cross-over study.

3. RECOMMENDATIONS

3.1 Alcohol

- Even though alcohol consumption may have protective effects on cardiovascular disease (CVD) through its positive effect on HDL-C, the consumption of alcohol for cardioprotective purposes as a public health measure is discouraged, given the deleterious effects it might have on blood pressure and other disease conditions. Alcohol, however, forms an important part of the lifestyle of many South Africans. Therefore, responsible light to moderate drinking (1 to 2 alcoholic drinks/day) for those who consume alcohol together with management of other CVD risk factors can be recommended. The consumption of alcohol is, however, not advised for pregnant and lactating women, former abusers, individuals with diseases or other conditions that might be adversely affected by this substance.

3.2 Sugar

- The message regarding sugar consumption for this westernised population and others that are moving towards westernised diets should be the positive one that sugar can be consumed in moderation (4-16%E) within diets that comply with recommended energy and micronutrient intakes.

- Because decreased sugar intake may have the effect of increasing fat intake, it is not necessary to avoid sugar in weight loss programmes. Women on low energy intake diets should, however, be careful not to displace nutrient and fibre-rich foods with foods containing large amounts of sugar.

- Sugar plays an important role in the palatability of food products and need not be avoided in the development of new healthy food products. The food industry should put in an effort towards the development of low fat products rather than low sugar or sugarless products, especially since reduction of fat intake appears to be a higher priority than the reduction of sugar intake in guidelines to prevent CVD (Wolmarans, 1999a; Wood *et al.*, 1998).

3.3 Lecithin

- Lecithin should not be prescribed by health professionals as an independent cholesterol-lowering agent, since it is ineffective and also expensive.

- The claiming of hypocholesterolaemic effects on lecithin brands should be forbidden, since it may mislead the uninformed public.

- The possible beneficial effect of sunflower oil on MPC levels needs to be further investigated, since it may represent a mechanism through which diet could lower risks of CVD.

3.4 High fibre *vs* low fibre meals on postprandial responses

- Since the possible detrimental effects of wheat bran demonstrated in this study were in contrast to the one study that could be found in which the effect of wheat fibre on postprandial lipemia was examined, these findings need to be confirmed by other postprandial studies before final conclusions can be made.

- The possible mechanisms responsible for these findings need to be examined by investigating the effect of wheat bran on intestinal digestion and absorption of dietary fat, activity of digestive enzymes, secretion of gastro-intestinal hormones and chylomicron catabolism.

- A positive, reassuring finding from this study was that the intake of normal physiological amounts of fat (30.5g fat/meal) did not activate factor VII, even though serum TG levels increased significantly.

3.5 Extruded dry beans

- Although the extruded dry bean product did not show the desired hypocholesterolaemic effects associated with dry beans, this should not negatively influence dietary recommendations regarding legume consumption. Dry beans is a rich and economical source of plant protein, complex carbohydrates, soluble and insoluble dietary fibre components and a variety of minerals and vitamins. It is also low in energy, fat and sodium content (Vorster and Venter, 1994). It therefore forms an important part of the hypocholesterolaemic diet.

- Apart from the above-mentioned benefits of dry beans, it might also have beneficial effects on PAI-1 levels, similar to the extruded product used in this study. These findings, however, need to be confirmed by other studies using dry beans *per se*.

- Processing techniques to develop alternative and convenient ways in which dry beans as a health food can be consumed, are encouraged. Care should be taken with the development of the extruded dry bean products so that acceptable products that fits into the hypocholesterolaemic diet are developed.

4 DISCUSSION OF MAIN FINDINGS AND RECOMMENDATIONS

A consensus meeting for the compilation of clinical guidelines for the diagnosis, management and prevention of the common dyslipidaemias in South Africa was held in August 1997. During this meeting a dyslipidaemia working group was formed. The author of this thesis represented the Nutrition Society of Southern Africa in this group. The Association for Dietetics of South Africa was tasked to develop clinical guidelines for the dietary management of people with dyslipidaemia. A technical paper (Wolmarans *et al.*, 1999a) provided the relevant scientific background for the dietary guidelines. This paper served as a discussion document for the dietary guideline working group, of which this author was also a member. The technical paper was amended and evaluated by an expert committee (Dr. JE Rossouw, National Institute of Health, USA; Dr. K Steyn, Chronic Diseases of Lifestyle Programme, Medical Research Council, Tygerberg, South Africa, and Prof. HH Vorster, Department of

Nutrition, Potchefstroom University for Christian Higher Education, Potchefstroom, South Africa). A position statement (Wolmarans *et al.*, 1999b) was written, based on the position paper, and was discussed by forty dieticians and nutritionists at a workshop, of which this author was the co-ordinator, during the 1998 Nutrition Congress (25-29 May 1998) at Sun City. The position statement was then summarised and translated into practical dietary guidelines (Wolmarans *et al.*, 1999c). These guidelines form part of *The diagnosis, management and prevention of the common dyslipidaemias in South Africa: clinical recommendations* that will be used by primary care practitioners (Marais *et al.*, 1999). Although these dietary guidelines were compiled for the management of dyslipidaemia, the importance of addressing the patient's total CVD risk profile is recognised. The guidelines will ensure optimal nutrition and are suitable for all dietary modifiable CVD risk factors. The compatibility of the main findings and recommendations following from the five studies reported in this thesis with the consensus dietary guidelines mentioned above will now be discussed:

- As recommended in this thesis, alcohol consumption is not encouraged, but for those who use alcohol, light to moderate drinking (1-2 alcoholic drinks/day) is recommended. The consumption of alcohol by persons with specific physiological or disease conditions is also discouraged.

- With regard to sugar intake, as in this thesis, moderate intake of sugar is recommended. The exception is in persons where TG concentrations are elevated. A reduction of sugar intake is recommended, but not forbidden. Data from this thesis did not allow recommendations regarding TG.

- As part of the dietary guideline group and after studying the literature regarding lecithin as a hypocholesterolaemic agent, this author could influence the group to include a guideline regarding lecithin supplementation. Because the literature could not provide conclusive evidence that lecithin lowers cholesterol levels, the supplementation with lecithin was not recommended. The results from this thesis support this guideline. The inclusion of lecithin as part of the dietary guidelines can be queried, since a wide range of dietary supplements exists and the inclusion of all these supplements may be unrealistic and impractical. Perhaps a separate guideline regarding dietary supplements in the prevention and management of

CVD must be compiled, with emphasis on anti-oxidants and folic acid (Willett, 1998). This thesis did not, however, address these micronutrients.

- Because of the inconsistent results shown in this thesis when high fibre and low fibre meals on postprandial responses were compared, conclusive recommendations could not be made and further research is needed. The dietary guidelines are, however, mostly based on results from studies that examined the effects of diet on fasting lipoprotein levels, probably because of the limited data available on postprandial responses. There is accordingly a need for more research regarding the effect of diet on postprandial responses, especially since it appears as if the effects on fasting levels and postprandial responses might not be necessarily in the same direction.

- In accordance with the recommendations from this thesis, the inclusion of dry beans as part of the hypocholesterolaemic diet is recommended and encouraged in the dietary guidelines.

5. CONCLUSION

In conclusion, results from the five studies reported in this thesis led to the formulation of recommendations regarding alcohol and sugar consumption and lecithin supplementation that can be used by Government, the food industry and health professionals for prevention of CVD. With regard to the effects of fibre-rich meals on postprandial lipaemia the results were inconclusive but recommendations for future research could be made. It was shown that the intake of physiological amounts of fat does not activate plasma factor VII levels. Although extruded dry bean intake did not have the desired hypocholesterolaemic effects associated with dry beans, it was shown that the extruded product could still form part of the CVD risk-reducing diet, since it may lower PAI-1 levels. It was also suggested that extrusion of dry beans is a valuable processing technique providing an alternative, convenient way in which dry beans can be consumed. It was suggested that care should be taken with the development of the extruded dry bean products to ensure that acceptable products that fit into the hypocholesterolaemic diet are developed.

This study therefore provided results that could be used in the formulation of dietary guidelines regarding the controversial issues of alcohol and sugar consumption. It clearly showed that despite several claims, lecithin has no independent hypocholesterolaemic effects. It further drew attention to the different effects dietary fibre may have on fasting and postprandial lipids, and on possible beneficial effects of functional food products on the haemostatic system. However, the major contribution of this study probably lies in the development of capacity to evaluate the physiological and biochemical effects of functional foods in a scientifically responsible way in well-designed controlled studies. Results from such studies could be used in future to define, describe and label the beneficial properties of such products in order to educate and inform the public to choose products and diets that will contribute to a lower risk of CVD.

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