THE EFFECT OF LEAN RED MEAT OR CHICKEN AND FISH, IN A PRUDENT DIET, ON THE LIPID METABOLISM OF HYPERCHOLESTEROLAEMIC SUBJECTS

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This thesis is dedicated to:

My mother and brother and
in loving memory of my father

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ABSTRACT

The aim of this clinical cross-over trial was to investigate the effect of two prudent diets, which differed only in the type of "meat" (lean red meat versus chicken without skin, and fish), on plasma lipids and lipoproteins of free-living subjects with age-related elevated plasma total cholesterol levels.

The study comprised of Phases 1 and 2, each with a baseline (Phase 1, three weeks; Phase 2, one week), a treatment (six weeks) and a post-treatment period (six weeks). There was a washout period of approximately two months between Phases 1 and 2. Seventy subjects, 36 men and 34 women, between the ages of 20 and 55 years and with elevated plasma total cholesterol levels were recruited for the study. Subjects were recruited from SANLAM, an insurance company, and from the Medical Research Council.

Subjects were matched for age and plasma total cholesterol, and randomly allocated to one of three groups; Study Groups 1 and 2, the treatment groups with 28 subjects in each group, and Study Group 3, reference group, with 14 subjects. In Phase 1, Study Group 1 followed a prudent diet with lean red meat (RMD) as the only "meat" in the diet, and Study Group 2 followed a prudent diet with chicken (without skin) and fish (CFD) as the only "meat" in the diet. In Phase 2 the diets were crossed-over. Each subject in the treatment group followed a prescribed diet based on their energy needs. Subjects received pre-packed lean red meat, chicken (without skin) and fish portions free of charge, but they were responsible for the remainder of their diet. The reference group followed their habitual diet (reference diet) throughout the study.

Measurements were taken at the start and end of the baseline period, after two weeks and at the end of the treatment period, and at the end of the post-treatment period. Blood pressure was measured and subjects were weighed. Blood samples, collected after an overnight fast, were analysed for plasma total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein $_1$ and $_2$ cholesterol (LDL_1-C, LDL_2-C), LDL_1-apolipoprotein B (apo B), LDL_2-apo B, very low density lipoprotein cholesterol (VLDL-C), VLDL-TAG, high density lipoprotein cholesterol (HDL-C) and HDL_3-C. HDL_2-C was calculated. The fatty acid composition (percentage) of plasma TAG and cholesteryl ester (CE) was also determined. Dietary data were collected by means of seven-

day dietary records during the baseline and the treatment periods. Food composition tables were used to analyse the dietary data for mean energy, dietary cholesterol and dietary fibre, and macronutrient (expressed as a percentage of total energy) intakes. The polyunsaturated to saturated fatty acid (P/S) ratio and the Keys dietary score were calculated. The Keys equation to predict the change in plasma TC as a result of a change in dietary fat and cholesterol intake was also calculated.

Fifty-two subjects, 21 from Study Group 1, 18 from Study Group 2 and 13 from Study Group 3, completed the cross-over study, but dietary data for only 50 subjects are reported.

Results showed that energy intakes, during the treatment periods were significantly lower than the energy prescribed. The subjects (97.1%) were of the opinion that they complied with 70% or more of the dietary prescription. Compliance with the dietary prescription, however, was better in Phase 1 than in Phase 2.

Mean total fat intake was less than 30 percent of energy (30%E) on the treatment diets except in Study Group 2 on the RMD (31%E). The P/S ratio of the diet was 0.93 on the RMD and approximately 1.5 on the CFD. In the reference group mean total fat intake remained above 30%E throughout the study and the P/S ratio of the diet varied between 0.64 and 0.70. Comparison of the treatment diets with the baseline diet showed that energy, total fat, saturated fat, monounsaturated fat and dietary cholesterol intakes were lower ($p = 0.0001$) on the RMD and the CFD than on the baseline diet. Protein and carbohydrate intakes ($p < 0.0005$) and the P/S ratio ($p = 0.0001$) were higher on the RMD and the CFD than on the baseline diet. On the RMD energy ($p = 0.0131$), total fat, saturated fat and monounsaturated fat ($p = 0.0001$) and dietary cholesterol ($p = 0.0012$) intakes were higher and the P/S ratio lower ($p = 0.0001$) than on the CFD. While energy, total fat, saturated fat and dietary cholesterol intakes were significantly higher on the reference diet than on the RMD or CFD, carbohydrate intake and the P/S ratio were significantly higher on the treatment diets.

The Keys dietary score, which gives an indication of the cholesterol elevating effect of the diet, was higher on the baseline ($p = 0.0001$) and the reference diets ($p < 0.001$) than on either the RMD or the CFD and it was also higher on the RMD ($p = 0.0001$) than on the CFD.

Lauric, myristic, palmitic and stearic acid intakes were higher ($p = 0.0001$) on the baseline diet than either on the RMD or the CFD. The intakes of myristic, palmitic and stearic acids were also higher ($p = 0.0001$) on the RMD than on the CFD and higher on the reference diet than on the treatment diets. The differences, however, were more pronounced for the CFD than for the RMD. Linoleic acid intake did not differ between the baseline diet and either the RMD or the CFD, and neither did it differ between the RMD and the CFD. Comparison of the RMD and the CFD showed that the intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intakes were higher ($p = 0.0001$) on the latter.

TC ($p = 0.0084$), LDL_1-C ($p = 0.0003$), LDL_2-C and LDL_1-apo B ($p = 0.0014$), and LDL_2-apo B ($p = 0.0001$) were lower while HDL-C:TC ($p = 0.0170$) was higher on the RMD than on the baseline diet. In agreement, TC ($p = 0.0001$), LDL_1-C ($p = 0.0003$), LDL_2-C ($p = 0.0001$), LDL_1-apo B ($p = 0.0133$) and LDL_2-apo B ($p = 0.0001$) were lower and HDL-C:TC ($p = 0.0025$) higher on the CFD than on the baseline diet. Comparison of the two treatment diets showed that only HDL-C ($p = 0.0498$) and diastolic blood pressure ($p = 0.0027$) were higher on the RMD than on the CFD. No significant differences in body mass, plasma lipids and lipoproteins or blood pressure were observed between the RMD and the reference diet. Body mass ($p = 0.0280$), LDL_2-C ($p = 0.0401$) and LDL_2-apo B ($p = 0.0211$), however, were lower on the CFD than on the reference diet.

Dietary intervention resulted in a change in plasma TC, which did not differ significantly from the value predicted by the Keys equation.

The percentage (%) of oleic acid in the plasma TAG and CE was significantly higher on the baseline diet than on either the RMD or the CFD. Oleic acid (%) in the TAG ($p = 0.0008$) and in the CE ($p = 0.0028$) was also significantly higher on the RMD than on the CFD. Stearic acid (%) in the CE was higher on the baseline diet than on the RMD ($p = 0.0001$) or on the CFD ($p = 0.0002$). Analysis for the direct treatment effect showed that stearic acid (%) in the TAG and CE was higher ($p<0.01$) on the RMD than on the CFD. The percentage of linoleic acid ($p<0.01$), EPA ($p = 0.0001$) and DHA ($p<0.0005$) and the EPA/arachidonic acid (EPA/AA) ratio ($p<0.0005$) in the plasma TAG and CE were significantly lower on the RMD than on the CFD. Despite a significant difference in the P/S ratio of the diets, the P/S ratio of the CE did not differ significantly between the RMD and the CFD. Comparison of the

reference and treatment diets showed that linoleic acid (%) and the P/S ratio of the TAG but not of the CE were significantly higher on the CFD than on the reference diet. The percentage of EPA, DHA and the EPA/AA ratio in the CE were lower ($p<0.05$) on the RMD than on the reference diet.

Cross-over analysis of the data indicated that there were, except for VLDL-TAG, palmitoleic acid and linolenic acid in the TAG, no significant differences for the first-order carry-over effects (baseline comparisons) or for the direct-by-period interaction effects.

In conclusion, this study showed that compared to a Western diet a prudent diet is effective in lowering the atherogenic lipoproteins without also lowering HDL-C levels and increasing TAG levels. No significant differences between the effect of a prudent diet containing either lean red meat or chicken (without skin) and fish on the atherogenic lipoproteins were observed. There were indications, however, that the CFD had a more favourable effect than the RMD on the fatty acid composition of plasma TAG and CE. It could be concluded that, depending on energy needs (7 500 to 14 000 kJ), a portion of lean red meat varying between 120 and 210 g (cooked weight) can be included in the prudent diet. The replacement of some of the lean red meat portions by as little as two fish meals per week, as well as the inclusion of chicken (without skin) in the diet could, however, be of additional benefit. An increase in the long chain polyunsaturated fatty acids and a decrease in the percentage of stearic acid in plasma TAG and CE may have beneficial effects in terms of thrombogenesis.

OPSOMMING

Die doel met hierdie kliniese oorkruis studie was om die effek van twee versigtige diëte, wat slegs verskil het in die tipe "vleis" (maer rooivleis versus hoender sonder vel en vis), op plasma lipiede en lipoproteïene van vry-lewende proefpersone met ouderdoms-verwante verhoogde plasma totale cholesterol te ondersoek.

Die studie het bestaan uit Fases 1 en 2, elk met 'n basislyn (Fase 1, drie weke; Fase 2, een week), 'n behandelings- (ses weke) en 'n na-behandelings periode (ses weke). Daar was 'n uitwas periode van ongeveer twee maande tussen Fases 1 en 2. Sewentig proefpersone, 36 mans en 34 vrouens, tussen die ouderdomme van 20 en 55 jaar en met verhoogde plasma totale cholesterolvlakke is gewerf vir die studie. Proefpersone is gewerf van SANLAM, 'n versekeringsmaatskappy en van die Mediese Navorsingsraad.

Proefpersone is gepaar vir ouderdom en plasma totale cholesterol, en ewekansig ingedeel in een van drie groepe: Studie Groepe 1 en 2, die behandelingsgroepe met 28 proefpersone in elke groep, en Studie Groep 3, die verwysingsgroep, met 14 proefpersone. In Fase 1 het Studie Groep 1 'n versigtige-dieet gevolg met maer rooivleis (RVD) as die enigste "vleis" in die dieet, en Studie Groep 2 het 'n versigtige-dieet gevolg met hoender (sonder vel) en vis (HVD) as die enigste "vleis" in die dieet. In Fase 2 is die diëte omgeruil. Elke proefpersoon in die behandelingsgroep het 'n voorgeskrewe dieet, wat gebasseer was op energiebehoefte, gevolg. Proefpersone het voorafverpakte maer rooivleis, hoender (sonder vel) en vis porsies gratis ontvang maar hulle was vir die res van hulle dieet verantwoordelik. Die verwysingsgroep het hulle gewone dieet (die verwysingsdieet) dwarsdeur die studie gevolg.

Metings is geneem aan die begin en einde van die basislyn, na twee weke en aan die einde van die behandelingsperiode, en aan die einde van die na-behandelingsperiode. Bloeddrukmetings is geneem en proefpersone is geweeg. Bloed is getrek na 'n oornag vas en is geanaliseer vir plasma totale cholesterol (TC), triasielgliserol (TAG), lae digtheid lipoproteïen $_1$ en $_2$ cholesterol (LDL_1-C, LDL_2-C), LDL_1-apolipoprotein B (apo B), LDL_2-apo B, baie lae digtheid lipoproteïen cholesterol (BLDL-C), BLDL-TAG, hoë digtheid lipoproteïen cholesterol (HDL-C) en HDL_3-C). HDL_2-C is bereken. Die vetsuursamestelling (persentasie) van plasma TAG en cholesteryl ester (CE) is ook bepaal. Dieetdata is versamel deur middel van sewe-dae dieetrekords gedurende

die basislyne en behandelings periodes. Voedselsamestellingstabelle is gebruik om die dieetdata te analiseer vir gemiddelede energie, dieetcholesterol en dieetvesel en makronutriënt (uitgedruk as a persentasie van total energie) innames. Die polionversadigde tot versadigde vetverhouding (P/V) en die Keys dieettelling is bereken. Die formule van Keys, om die verandering in plasma totale cholesterol te voorspel op grond van 'n verandering in dieetvet en cholesterol inname, is ook bereken.

Twee-en-vyftig proefpersone, 21 van Studie Groep 1, 18 van Studie Groep 2 en 13 van Studie Groep 3 het die oorkruisstudie voltooi maar dieetdata vir slegs 50 proefpersone word gerapporteer.

Resultate het getoon dat energie innames gedurende die behandelingsperiodes betekenisvol laer was as die energie wat voorgeskryf is. Die proefpersone (97.1%) was van mening dat hulle die dieetvoorskrifte 70% of meer gevolg het. Navolging van die dieetvoorskrifte was egter beter in Fase 1 as in Fase 2.

Gemiddelde totale vetinname was minder as 30 persent van energie (30%E) op die behandelingsdieet behalwe in Studie Groep 2 op die RVD (31%E). Die P/V verhouding van die dieet was 0.93 op die RVD en ongeveer 1.5 op die HVD. In die verwysingsgroep het die totale vetinname bo 30%E gebly tydens die studie en die P/V verhouding van die dieet het gewissel tussen 0.64 en 0.70. In vergelyking met die basislyn was energie, totale vet, versadigde vet, mono-onversadigde vet en dieetcholesterol innames laer ($p = 0.0001$) op die RVD en die HVS. Proteïen en koolhidraat innames ($p < 0.0005$) en die P/V verhouding ($p = 0.0001$) was hoër op die RVD en die HVD as op die basislyn dieet. Op die RVD was die energie ($p = 0.0131$), totale vet, versadigde vet en mono-onversadigde vet ($p = 0.0001$), en dieetcholesterol ($p = 0.0012$) innames hoër en die P/V verhouding ($p = 0.0001$) laer as op die HVD. Terwyl energie, totale vet, versadigde vet en dieetcholesterol innames betekenisvol hoër was op die verwysingsdieet as op die RVD of die HVD, was koolhidraatinname en die P/V verhouding betekenisvol hoër op die behandelingsdiëte.

Die Keys dieettelling, wat 'n aanduiding gee van die cholesterol verhogende effek van die dieet was hoër op die basislyn- ($p = 0.0001$) en die verwysingsdiëte ($p < 0.001$) as op of die RVD of die HVD en dit was ook hoër op die RVD as op die HVD.

Laurien-, miristien-, palmitien- en steariensuur innames was hoër ($p = 0.0001$) op die basislyn dieet as op of die RVD of die HVD. Die innames van miristien-, palmitien- en steariensuur was ook hoër ($p = 0.0001$) op die RVD as op die HVD en hoër op die verwysingsdieet as op die behandelingsdiëte. Die versille was egter meer uitgesproke in die HVD as op die RVD. Linoleïensuur inname het nie verskil tussen die basislyn dieet en die RVD of die HVD en dit het ook nie verskil tussen die RVD en die HVD. Vergelyking van die RVD met die HVD het getoon dat die inname van eicosapentaenoësuur (EPA) en dokosahexaenoësuur (DHA) hoër was ($p = 0.0001$) op laasgenoemde.

TC ($p = 0.0084$), LDL_1-C ($p = 0.0003$), LDL_2-C en LDL_1-apo B ($p = 0.0014$), en LDL_2-apo B ($p = 0.0001$) was laer terwyl HDL-C:TC ($p = 0.0170$) hoër was op die RVD as op die basislyn dieet. Ooreenstemmend was TC ($p = 0.0001$), LDL_1-C ($p = 0.0003$), LDL_2-C ($p = 0.0001$), LDL_1-apo B ($p = 0.0133$) en LDL_2-apo B ($p = 0.0001$) laer en HDL-C:TC ($p = 0.0025$) hoër op die HVD as op die basislyn dieet. Vergelyking van die twee behandelingsdiëte het getoon dat slegs HDL-C ($p = 0.0498$) en diastoliese bloeddruk ($p = 0.0027$) hoër was op die RVD as op die HVD. Geen betekenisvolle verskille in liggaamsgewig, plasmalipiede en lipoproteïene of bloeddruk is waargeneem tussen die RVD en die verwysingsdieet nie. Liggaamsgewig ($p = 0.0280$), LDL_2-C ($p = 0.0401$) en LDL_2-apo B ($p = 0.0211$) was egter laer op die HVD as op die verwysingsdieet.

Dieetintervensie het 'n verandering in plasma TC tot gevolg gehad en die waardes het in die algemeen nie betekenisvol verskil van die waardes wat deur die Keys formule voorspel is nie.

Die persentasie (%) van oleïensuur in die plasma TAG en CE was betekenisvol hoër op die basislyn dieet as op of die RVD of die HVD. Oleïensuur (%) in die TAG ($p = 0.0008$) en in die CE ($p = 0.0028$) was ook betekenisvol hoër op die RVD as op die HVD. Steariensuur (%) in die CE was hoër op die basislyndieet as op die RVD ($p = O.0001$) of op die HVD ($p = 0.0002$). Analise van die direkte behandelingseffek het getoon dat steariensuur (%) in die TAG en CE hoër ($p<0.01$) was op die RVD as op die HVD. Die persentasie van linoleïensuur ($p<0.01$), EPA ($p = 0.0001$) en DHA ($p<0.0005$) en die EPA/aragidoonsuur (EPA/AS) verhouding ($p<0.0005$) in die plasma TAG en CE was betekenisvol laer op die RVD as op die HVD. Ten spyte van 'n verskil in die P/V verhouding van die diëte het die P/V verhouding van die CE nie betekenisvol verskil tussen die RVD en die HVD. Vergelyking van

die verwysingsdieet met die behandelingsdiëte het getoon dat linoleïensuur (%) en die P/V verhouding van die TAG en CE betekenisvol hoër was op die HVD as op die verwysingsdieet. Die persentasie EPA, DHA en die EPA/AS verhouding in die CE was laer ($p<0.05$) op die RVD as op die verwysingsdieet.

Oorkruis analise van die data het getoon dat met die uitsondering van BLDL-TAG, palmitoleïensuur en linoliniensuur in die TAG, was daar geen betekenisvolle verskille in die eerste-orde oordrag effekte (basislyn vergelykings) of vir die direkte-per-periode interaksie effekte.

Opsommend het hierdie studie getoon dat in vergelyking met 'n Westerse dieet was 'n versigtige-dieet effektief in die verlaging van die aterogeniese lipoproteïene sonder om ook die HDL-C vlakke te verlaag of die TAG vlakke te verhoog. Geen betekenisvolle verskille tussen die effekte van die versigtige-dieet wat maer rooivleis of hoender (sonder vel) en vis bevat het op die aterogeniese lipoproteïene is waargeneem nie. Daar was egter 'n aanduiding dat die HVD 'n meer gunstige effek gehad het op die vetsuursamestelling van die plasma TAG en CE as die RVD. Daar kan tot die gevolgtrekking gekom word dat, afhangende van energie behoeftes (7 500 kJ tot 14 000 kJ) 'n porsie maer rooivleis wat wissel tussen 120 en 210 g (gaar gewig) ingesluit kan word in die versigtige-dieet. Die vervanging van 'n gedeelte van die maer rooivleis in die dieet met so min as twee vismaaltye per week sowel as hoender (sonder vel) kan egter addisionele voordele inhou. 'n Verhoging van die langketting polionversadigde vetsure en 'n verlaging van stearіensuur in die plasma TAG en CE mag voordele inhou ten opsigte van trombogenese.

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ABBREVIATIONS

AA	arachidonic acid
AHA	American Heart Association
Apo	apolipoprotein
ASCN/AIN	American Society for Clinical Nutrition/ American Institute of Nutrition
BMI	body mass index
CARDIA	Coronary Artery Risk Development in Young Adults study
CE(s)	cholesteryl ester(s)
CERSA	Centre for Epidemiological Research in Southern Africa
CETP	cholesterol ester transfer protein
CFD	chicken-fish diet
CHD	coronary heart disease
COMA	Committee of Medical Aspects
CORIS	Coronary Risk Factor Intervention Study
CRISIC	Coronary Risk factor Study in the coloured population of the Cape Peninsula
DGLA	dihomo-gamma-linolenic acid
DHA	docosahexaenoic acid
DRV	Dietary Reference Values
EC	esterified cholesterol
EDTA	ethylenediamine-tetra-acetic acid
e.g.	*exempli gratia* (for example)
EPA	eicosapentaenoic acid
EURAMIC	European Countries and Israel study
FCT	Food Composition Tables
FH	familial hypercholesterolaemia
FQM	Food Quantities Manual
GC	gas chromatograph
GLA	gamma-linolenic acid
HDL	high density lipoprotein
HDL-C	high density lipoprotein cholesterol
HLP	hyperlipoproteinaemia(ic)

HMAC	Health Matters Advisory Committee
HTGL	hepatic triacylglycerol lipase
HyperapoB	hyperapoB-lipoproteinaemia
HyperTAG	hypertriacylglycerolaemia
IDL	intermediate density lipoprotein
i.e.	*id est* (that is)
kJ	kilojoules
kcal	kilocalories
kg	kilogram
LCAT	lecithin:cholesterol acyltransferase
LDL	low density lipoprotein
LDL-C	low density lipoprotein cholesterol
Lp(a)	lipoprotein(a)
LPL	lipoprotein lipase
LRC-CPPT	Lipid Research Clinics Coronary Primary Prevention Trial
mL	milliliter
$MnCl_2$	manganese chloride
MRC	Medical Research Council
MRFIT	Multiple Risk Factor Intervention Trial
MUFAs	monounsaturated fatty acid(s)
n-3	omega-three
n-6	omega-six
NACNE	National Advisory Committee on Nutrition Education
NCEP	National Cholesterol Education Program
NIH	National Institutes of Health
NRIND	National Research Institute for Nutritional Diseases
NSP	non-starch polysaccharides
PL	phospholipids
P/S ratio	polyunsaturated fatty acid to saturated fatty acid ratio
PUFA(s)	polyunsaturated fatty acid(s)
RDA(s)	Recommended Dietary Allowance(s)
RMD	red meat diet
SAS	statistical analysis system

SD	standard deviation
SFA(s)	saturated fatty acid(s)
TLC	thin layer chromatography
TAG	triacylglycerol
TC	total cholesterol
UK	United Kingdom
USA	United States of America
VLDL	very low density lipoprotein
VLDL-C	very low density lipoprotein cholesterol
VLDL-TAG	very low density lipoprotein triacylglycerol
WHO	World Health Organisation
%E	percent of energy

CHAPTER 1

INTRODUCTION

1.1 Background and motivation

In the Seven Countries Study serum cholesterol as well as coronary heart disease (CHD) deaths and infarctions showed a high positive correlation (0.89 and 0.84 respectively) with the percentage of energy from saturated fatty acids (SFAs) (Keys, 1970). The prevalence of ischaemic or CHD is also high among especially white South Africans (Department of National Health and Population Development, 1992). This population group is known to follow a Western type diet high in total and saturated fat (Wolmarans *et al.*, 1988). Mortality rates reported showed that in 1989 the standardised rates per 100 000 were 159 for men and 102 for women (Department of National Health and Population Development, 1992). The coloured population has not been studied extensively, but the Coronary Risk Factor Study in the Cape Peninsula (CRISIC study) indicated that risk factors for CHD are a major problem in this community (Steyn *et al.*, 1985). A high prevalence of CHD risk factors has also been reported for Indians living in Durban (Seedat *et al.*, 1990). Although the prevalence of CHD is still low in black South Africans an increased incidence is predicted by many authors (Steyn *et al.*, 1991; Mollentze *et al.*, 1995).

The prevalence of hypercholesterolaemia (serum cholesterol $\geq$5.7 mmol/L), one of the main risk factors for CHD, is especially high among white South Africans and the age-adjusted figures reported for men and women were 45.3% and 46.7% respectively (Rossouw *et al.*, 1983). In coloured and Indian South Africans the prevalence of hypercholesterolaemia was also found to be high (Steyn *et al.*, 1985; Seedat *et al.*, 1990). In coloured South Africans the age-adjusted figures were 33.8% for men and 33.3% for women, while it was 39.6% for Indian men and 29.8% for Indian women (Steyn *et al.*, 1985; Seedat *et al.*, 1990). Mollentze *et al.* (1995) reported moderate-risk hypercholesterolaemia (action limits for serum cholesterol - Heart Foundation of Southern Africa) in 34% of QwaQwa and 44.8% of Mangaung rural black South African men 25-34 years of age. A study on urban blacks, however, showed that only 15.4%

of men and 23.5% of women were in the moderate risk category of hypercholesterolaemia (Steyn *et al.*, 1991).

Familial hypercholesterolaemia (FH) was found to be especially prevalent among Afrikaans-speaking white South Africans (Seftel *et al.*, 1980; Jooste, Benadé & Rossouw, 1986; Steyn *et al.*, 1989). Jooste, Benadé & Rossouw (1986) found a prevalence ratio of 1:87 in white South Africans studied in three South-Western Cape communities and estimated that the prevalence could even be as high as 1:47. This is much higher than the figure of 1 in 500 persons reported for the prevalence of heterozygotes among European, American and Japanese populations (Goldstein & Brown, 1983).

The diets of those in whom the prevalence of CHD is high (Wolmarans *et al.*, 1988; Langenhoven *et al.*, 1988a) were also characterised by a total fat intake of approximately 35 percent of energy (35%E) or more and a saturated fat intake of approximately 12%E or more. Indian South Africans also consume a diet containing approximately 34%E from fat but the polyunsaturated fatty acid (PUFA) to SFA ratio (P/S ratio) of their diet is high and thus more favourable (Wolmarans *et al.*, unpublished data). Black South Africans, in whom the prevalence of CHD and hypercholesterolaemia is low, has a total fat intake of approximately 26%E, while their SFA intake is approximately 9%E (Bourne *et al.*, 1993). Dietary cholesterol intakes in the South African population groups studied varied between 233 mg/day (range 174 - 265 mg/day) in those who follow a prudent diet (Bourne *et al.*, 1993) and 362 mg/day (range 243 - 509 mg/day) in those on a Western type diet (Wolmarans *et al.*, 1988).

Dietary intervention is the first step in the treatment of hypercholesterolaemia (Adult Treatment Panel II, 1994) and this should be the case whether hypercholesterolaemia is ascribed to genetic or environmental factors. Many countries have guidelines for the prevention of CHD (Mann *et al.*, 1993; Noticeboard, 1991; Scientific Review Committee, 1990; American Heart Association, 1988). The diet recommended to prevent CHD is also known as the prudent diet. In 1989 dietary guidelines for the prevention of CHD were formulated for South Africa (Diet Consensus Panel, 1989). The guidelines for most of the countries were formulated for the general population. New Zealand also formulated separate dietary guidelines for those with identified hypercholesterolaemia (Mann *et al.*, 1993). However, in South Africa the dietary guidelines have a population approach rather than focusing only on those with identified hypercholesterolaemia.

Dietary guidelines formulated for South Africa recommend *inter alia* the following: total fat intake should be less than 30%E, saturated fat intake not more than 10%E, polyunsaturated fat intake should not exceed 10%E; dietary cholesterol intake <300 mg per day; fibre intake 20-30 g per day (Diet Consensus Panel, 1989). These dietary guidelines should, however, be translated into foods for those who need to follow a cholesterol-lowering diet. In order to meet the dietary guideline of less than 30%E from total fat and less than 10%E from saturated fat it is recommended that people should restrict their intake of animal fat which is the main source of saturated fat in the Western diet. Since red meat is seen as an important source of saturated fat the restriction or exclusion of red meat from the diet is often recommended in order to lower plasma cholesterol levels. This could, however, prove to be unrealistic for South Africans with hypercholesterolaemia who follow a Western diet. It was shown that red meat contributed to between 30% and 39% of the saturated fat intake of South Africans following a Western diet (Wolmarans *et al.*, 1989). Moreover, blacks in South Africa are in a process of changing their eating patterns, *inter alia* to include more meat in their diet. According to Walker, Labadarios & Glatthaar (1995) it would be unrealistic to expect developing populations in South Africa not to alter their trend towards a more palatable Western diet which contains much more meat.

The relationship between red meat consumption and CHD is not clear. In 1985 the per capita consumption of red meat (beef and mutton) by white South Africans was 54.8 kg (Personal communication: Mr Anton Meiring, South African Meat Board, 1993). The prevalence of CHD is also very high in this group. In the same year the age-specific mortality rate (per 100 000) for CHD in white South Africans was 178 (Department of National Health and Population Development, 1992). However, in data published for the European Community no relation was shown between meat (beef, lamb and goat) consumption, in kilogram per head per anum, and CHD mortality (Ulbricht & Southgate, 1991). Greece, one of the countries with the lowest CHD rates, had the highest red meat consumption. A study in vegetarians, however, showed that a daily isocaloric addition of 250 g beef to the diet increased total cholesterol (TC) by 19% (Sacks *et al.*, 1981). Data on cholesterol concentrations suggested that, in Britain, the prevalence of CHD may be 24% lower in lifelong vegetarians than in meat eaters (Thorogood *et al.*, 1987). It is not clear if red meat *per se* is responsible for this effect. The diet of vegetarians might contain more fibre, omega-three (n-3) fatty acids such as linolenic acid (C18:3 n-3) and antioxidants, known to protect against CHD. In addition, there might also be other differences in lifestyle between vegetarians and non-vegetarians e.g. smoking habits.

There are indications in the literature that lean red meat can be included in lipid-lowering diets. Watts *et al.* (1988) showed that the plasma TC concentration of hyperlipidaemic patients could be lowered significantly by lipid-lowering diets which included a 180 g portion of lean red meat per day. Although the plasma low density lipoprotein cholesterol (LDL-C) concentration also decreased, very low density lipoprotein triacylglycerol (VLDL-TAG) and high density lipoprotein cholesterol (HDL-C) concentrations did not change significantly. O'Dea *et al.* (1990) also showed that lean beef could be included in cholesterol-lowering diets and that it was the beef fat and not the lean beef itself which has a cholesterol-elevating effect. When diets containing beef and pork were compared with diets of poultry and fish, no differences in TC levels could be shown in free-living normolipidaemic subjects (Flynn *et al.*, 1981, 1982).

There is often controversy in the media and sometimes also among health professionals on how many times per week red meat should be prescribed to those with hypercholesterolaemia. In order to answer this question a study was undertaken to establish how often South African medical doctors prescribe the consumption of red meat per week for their patients with hypercholesterolaemia (Wolmarans *et al.*, unpublished data). The response rate on a questionnaire mailed to 745 doctors was 30.6%. Results showed that 66.7% of the physicians and 61.6% of the general practitioners do not recommend the exclusion of red meat from the diet. The rest recommended that red meat should always, usually or sometimes be excluded from the diet. Approximately 3% of the medical doctors recommended that a small portion of lean red meat can be eaten daily.

It is often stated that there are no "bad" foods (except those containing chemical or bacteriologic toxins) but that there are "bad" or "good" diets (Anon, 1988). Whether red meat should be regarded as a "bad" food for those with hypercholesterolaemia could therefore be questioned.

1.2 Main aim of the study

The **aim of this study** was to investigate the effect of two prudent diets, which differed only in the type of "meat" (lean red meat versus chicken, without skin, and fish), on plasma lipids and lipoproteins of free-living subjects with age-related elevated plasma cholesterol levels.

1.3 Specific objectives of the study

* to compile treatment diets which could be followed outside the clinical setup;
* to evaluate dietary compliance;
* to report on dietary intake during the study;
* to evaluate the effect of treatment diets on plasma lipids and lipoprotein profiles as well as the fatty acid composition of plasma triacylglycerol (TAG) and cholesteryl ester (CE).

1.4 Hypothesis

The hypothesis tested in this study is that two prudent diets, which differed only in the type of "meat" (lean red meat versus chicken, without skin, and fish), will not differ in their effect on plasma lipids and lipoprotein levels of free-living subjects with age-related elevated plasma cholesterol levels. This hypothesis was tested in a study with a cross-over design in which there was careful control of the possible effects of other dietary variables than type of meat.

1.5 Structure of the thesis

In this thesis, the introductory chapter is followed by a review of the relevant literature (Chapter 2). In Chapter 3 the study design and all methods used are discussed while in Chapter 4 results on dietary compliance and dietary intake will be reported and discussed. Results on plasma lipids and plasma lipoproteins will be reported and discussed in Chapter 5. In Chapter 6 results on the fatty acid composition of plasma TAG and CE will be given and discussed. The results of this study will be summarised in Chapter 7, conclusions will be drawn from the results and recommendations will be made.

CHAPTER 2

LITERATURE REVIEW

2.1 Lipids and lipoproteins as risk factors for coronary heart disease

2.1.1 Introduction

Total cholesterol, phospholipids (PL) and TAG are fat-like substances (lipids) found in the blood. Cholesterol forms an essential part of cell membranes and it is also a precursor of bile acids and steroid hormones. Total cholesterol, PL and TAGs which are not soluble in water are bound to specific proteins called apoproteins to form lipoproteins, which are water soluble complexes (Assmann, 1982).

The lipoproteins in plasma are chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). The main characteristics of the plasma lipoproteins are summarised in Table 2.1 and in Figure 2.1 a schematic presentation of lipoprotein metabolism is given.

High plasma TC, especially high LDL-C and probably also high TAG levels, are risk factors for the development of coronary heart disease. Low HDL-C levels may also be a risk factor for CHD.

In this section the relationship of plasma lipids and lipoproteins with CHD will be discussed. This will be followed by a section on the effects of dietary substances on circulating lipids and lipoproteins and dietary guidelines for the prevention of CHD. Red meat as a part of a lipid lowering diet will also be discussed.

Table 2.1. Characteristics of plasma lipoproteins (table modified from Mahley, 1991; Assman, 1982; Ginsberg, 1990)

Class	Density of flotation (g/mL)	Lipids	Apoproteins	Origin	Physiological role
Chylomicrons	$d < 0.95$	TAG* (86-94%) PL† (3-8%) CE# (1-3%) FC§ (0.5-1%)	A-I, A-IV, B-48, {C-I, C-II, C-III, E - *transferred from HDL*} (1-2%)	Formed in the mucosal cells of the small intestine	Exogenous TAG transport
Chylomicron remnants	$d < 0.95$	TAG cholesterol	B-48 B-100 E	Formed from chylomicrons after TAG hydrolysis by lipoprotein lipase.	Delivery of cholesterol to the liver. May be atherogenic.
Very low density lipoprotein (VLDL)	$d < 1.006$	TAG (55-65%) PL (12-18%) CE (12-14%) FC (6-8%)	B-100, C-I, C-II, C-III, E (5-10%)	Formed in the liver. Assembled in hepatocytes.	Transport of endogenous TAG
Intermediate density lipoprotein (IDL)	$d = 1.006$-1.019	TAG (20-50%) CHOL¶ (20-40%) PL (15-25%)	B-100, C-I, C-II, C-III, E	Formed from VLDL after hydrolysis of TAG by lipoprotein lipase	Precursor of low density lipoprotein (some IDL taken up by the liver)
Low density lipoprotein (LDL)	$d = 1.019$-1.063	CE (35-40%) PL (20-25%) TAG (8-12%) FC (5-10%)	B-100 (20-24%)	Formed from VLDL and IDL after hydrolysis of TAG	Major lipoprotein for the transport of cholesterol to the peripheral tissues. Major lipoprotein associated with coronary heart disease.
High density lipoprotein (HDL)	$d = 1.063$-1.21	PL (20-30%) CE (14-18%) TAG (3-6%) FC (3-5%)	A-I, A-II, A-IV, C-I, C-II, C-III, E (45-50%)	Nascent HDL particles produced by the liver and small intestine, then fully developed in the plasma by acquiring surface components from chylomicrons and VLDL	Reverse cholesterol transport

*TAG - Triacylglycerol; †PL - Phospholipids; #CE - cholesteryl esters; § FC - free cholesterol; ¶CHOL - total cholesterol (Ginsberg, 1990)

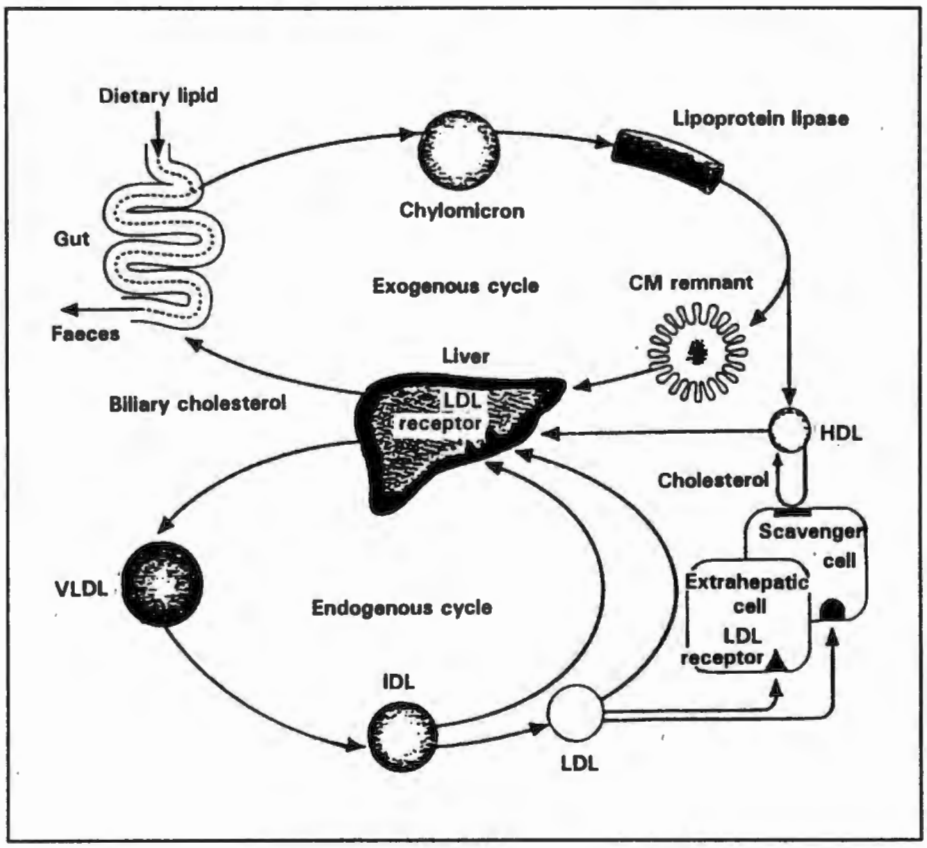

Figure 2.1 Endogenous and exogenous cycles of lipoprotein metabolism

(Adapted from: *Clinical Biochemistry*, by Allan Gaw, Robert A Cowan, Denis St J O'Reilly, Michael J Steward, James Shepherd. (Edinburgh: Churchill Livingstone 1995, p163, and printed in *Lancet* (1995) vol 346, p626)

2.1.2 Plasma total cholesterol and coronary heart disease

2.1.2.1 Epidemiology

Epidemiological studies have shown that populations with high plasma TC levels have a high prevalence of CHD (Keys, 1970). In the *Second Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II)* the large body of evidence which supports the relationship between TC and CHD between and within populations is discussed (Adult Treatment Panel II, 1994). Also in South Africa a high prevalence of hypercholesterolaemia has been reported in those populations in whom the prevalence of CHD is high (Rossouw *et al.*, 1983; Steyn *et al.*, 1985; Seedat *et al.*, 1990).

It is generally accepted that high blood cholesterol levels are causally related to atherosclerosis and increased risk for CHD (American Heart Association, 1990). Assmann (1982) summarised findings of the American Heart Association Pooling Project and showed that the relationship between TC and CHD was essentially linear. The United States Consensus Conference as well as the European Atherosclerosis Society see cholesterol as playing a causal role in CHD (Brook & Rifkind, 1989). The British Atherosclerosis Society, however, has a slightly more conservative viewpoint and sees the relationship between serum TC and CHD as continuous and curvilinear (Brook & Rifkind, 1989).

2.1.2.2 Plasma total cholesterol and the risk of coronary heart disease

Although high plasma TC levels ($\geq$6.2 mmol/L) are associated with a high prevalence of CHD, CHD also occurs in people with cholesterol levels lower than 6.2 mmol/L (Dalen, 1991). There are indications that the relationship between levels of TC and total mortality is J-shaped and that there are higher total mortality rates with high TC levels as well as with quite low levels of TC $<$3.6 mmol/L ($<$140 mg/dL) (Adult Treatment Panel II, 1994). A progressive increase in CHD death was observed in the Multiple Risk Factor Intervention Trial (MRFIT) in men with a TC level above 4.68 mmol/L (181 mg/dL) while in those with a cholesterol level above 6.54 mmol/L (253 mg/dL) the relative risk of CHD death was 3.7 that of men in the bottom quartile (Hulley, 1988). The association between elevated serum TC and increased CHD seems to start at a TC level of 4.65 mmol/L (180 mg/dL) and the steady increase in risk is particularly observed above the level of 5.2 mmol/L (200 mg/dL) (American Heart Association, 1990; Dalen, 1991). Total cholesterol levels between 4.1 mmol/L (160 mg/dL) and 5.1 mmol/L (199 mg/dL) seem to be the levels between which the lowest total mortality occurs in men (Adult Treatment Panel II, 1994). In order to reduce the incidence of CHD there is no reason to lower TC levels to less than 4,14 mmol/L (160 mg/dL) (Adult Treatment Panel II, 1994).

2.1.2.3 Reference values for total cholesterol

In South Africa age-standardised cut-off points for moderately and severely elevated TC levels have been defined (Rossouw *et al.*, 1988) and are used to classify people as moderately or severely at risk of CHD in terms of serum TC levels (Figure 2.2).

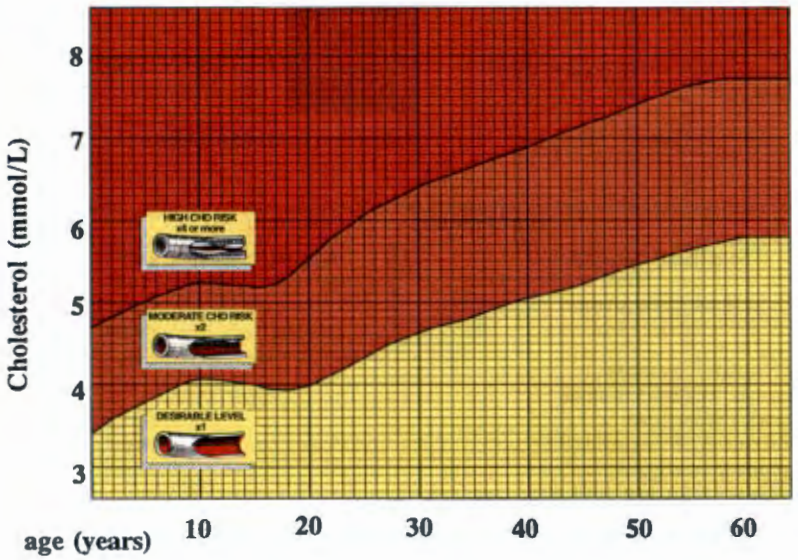

Figure 2.2. Cholesterol action limits (Reference: Heart Foundation of Southern Africa)

Reference values for plasma lipids as defined internationally are given in Table 2.2.

Table 2.2. Reference values for plasma lipids

	TC*	LDL-C†	HDL-C§	TAG¶**
		mmol/L		
Desirable	<5.2	<3.4	≥1.56	2.3
Borderline	5.2-6.2	3.4-4.1		2.3-4.5
High risk	≥6.2	>4.1	<0.90	4.5-11.3

* Total cholesterol;
† Low density lipoprotein cholesterol;
§ High density lipoprotein cholesterol;
¶ Triacylglycerol;
** Definitions based on cholesterol management and does not give categories of hyperTAG.
Reference: Adult Treatment Panel II, 1994.

Many regard a serum TC level of 5.2 mmol/L as desirable for adults (Brook & Rifkind, 1989; Schaefer, 1993; Study Group, European Atherosclerosis Society, 1987) while the US Consensus

Conference and the European Atherosclerosis Society recommend a level of 4.7 mmol/L for persons younger than 30 years of age.

2.1.2.4 Plasma total cholesterol and the prevention of coronary heart disease

It is clear from the literature that the risk of CHD is decreased by the lowering of plasma TC levels (American Heart Association, 1990; Dalen, 1991; Adult Treatment Panel II, 1994). Intervention studies such as The Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT) and the Oslo study have shown that the incidence of CHD can be lowered by decreasing plasma TC levels (Lipid Research Clinics Program, 1984; Hjermann *et al.*, 1981). Dietary treatment or dietary plus cholestyramine treatment of men with CHD significantly reduced the frequency of total cardiovascular events (Watts *et al.*, 1992).

Overall progression was retarded and overall regression of coronary artery disease was increased with dietary treatment or with diet plus cholestyramine. Plasma TC, LDL-C, TC/HDL-C ratio and LDL:HDL ratio decreased significantly on dietary treatment and diet plus cholestyramine, although more so in the latter. It has been estimated that with a 1% reduction in serum TC the risk of CHD events will be reduced by approximately 2% (American Heart Association, 1990; Adult Treatment Panel II, 1994). Although this may be relevant for the prediction of CHD in populations it may not be applicable in individuals because of fluctuations in serum cholesterol concentrations. Mogadam *et al.* (1990) have showed that in 75% of the 20 subjects they studied, aged 22 to 63 years, serum TC levels fluctuated by more than $\pm$20% on retesting over a four-week period. Small changes of 5%-10% in serum TC may not be clinically meaningful for an individual (Mogadam *et al.*, 1990).

Factors such as age and genetics influence plasma TC levels but diet plays a very important role (American Heart Association, 1990). Although the former cannot be changed the latter is a modifiable risk factor. Not only are those with high blood cholesterol levels at risk for CHD, but people with mild or moderately elevated cholesterol levels are also at risk. Strategies to lower the prevalence of CHD should therefore not only be aimed at the high-risk individual, but also to the population. A population-based strategy should therefore be followed.

2.1.3 Triacylglycerol and very low density lipoprotein and coronary heart disease

2.2.3.1 General information

In Table 2.1 the characteristics of chylomicrons and VLDL are summarised. The main carriers of TAG are chylomicrons and VLDL. Chylomicrons are produced in the intestine while TAG-rich VLDL is produced by the liver (American Heart Association, 1984). The chylomicrons first pass into the lymph after synthesis in the intestinal wall and enter the blood through the thoracic duct (Assmann, 1982; American Heart Association, 1984; Kris-Etherton *et al.*, 1988). The synthesis and catabolism of VLDL and LDL represent the endogenous transport of fat (Kris-Etherton *et al.*, 1988) and are schematically portrayed in Figure 2.1.

Apo C-II is the cofactor for the enzyme lipoprotein lipase (LPL) which is responsible for the catabolism of the TAG-rich lipoproteins, namely chylomicrons and VLDL (Thompson, 1989). A deficiency of apo C-II is responsible for severe hypertriacylglycerolaedemia (hyperTAG) (Thompson, 1989). Apo C-III inhibits LPL and is therefore most likely involved in the control of TAGs (Tan *et al.*, 1993). There are indications that VLDL remnants are atherogenic (Assmann, Gotto & Paoletti, 1991) because their clearance by the liver is impaired (Tan *et al.*, 1993). The larger TAG-rich VLDL lipoproteins are less atherogenic than the smaller cholesterol-enriched VLDL lipoproteins (Adult Treatment Panel II, 1994).

2.1.3.2 Triacylglycerol as a risk factor for coronary heart disease

Elevated TAG levels are a risk factor for CHD but probably not to the same extent as elevated plasma TC levels (Adult Treatment Panel II, 1994; Gordon, 1990). It is not yet clear whether there is a causal relationship between hyperTAG and CHD (NIH Consensus Conference, 1993) although there are studies which showed that TAG was independently related to CHD risk (Bainton *et al.*, 1992). In individuals with an LDL:HDL ratio of >5 it was shown that TAG is associated with CHD (Tan *et al.*, 1993). HDL-C and TAG vary in a reciprocal manner and the association between raised TAG levels and CHD might be due to low HDL-C levels (Gordon, 1990; Thompson, 1989; Assmann, 1982). Low HDL-C levels are known to be a risk factor for CHD (Gordon, 1990). In a study on men and women of Taipei ($n = 440$) and Framingham ($n = 428$) aged 40-59 years, a significant inverse correlation between HDL-C and the log of TAG levels was found (Lyu *et al.*, 1993). There are indications that elevated TAG levels may only be a risk factor for CHD in women (Castelli, 1986).

In a prospective study on 21 520 men aged 35-64 years TAGs were no longer significantly associated with the risk of CHD after adjustment for apolipoprotein B (apo B). However, TAGs were still associated ($p = 0.006$) with CHD after adjustment for TC, apo A-I (or HDL-C) and apo(a) (Wald *et al.*, 1994). Postprandial but not fasting TAGs were found to be an independent risk factor for CHD (Patsch *et al.*, 1992). High LPL activity will reduce postprandial lipaemia and high HDL_2 levels are a reflection of the effective catabolism of TAG-rich lipoproteins (Gotto *et al.*, 1991). The findings of a case-control study on 61 men with severe CHD and 40 control subjects indicated that the negative association between HDL-C and CHD originates in part from the positive association between postprandial plasma TAGs and CHD (Patsch *et al.*, 1992).

TAG-rich lipoproteins play an important role in the thrombotic process (Tan *et al.*, 1993). HyperTAG may influence CHD through its role in coagulation. An increase in TAGs are associated with an increase in factor VII (Folsom *et al.*, 1991) while tissue-type plasminogen activator and plasminogen activator inhibitor are also associated with hyperTAG (Mussoni *et al.*, 1992).

2.1.3.3 Reference values for triacylglycerol

Reference values for TAG levels are given in Table 2.2. The risk of pancreatitis is raised with a TAG level above 11,3 mmol/L (NIH Consensus Conference, 1993).

2.1.3.4 Triacylglycerol and the prevention of coronary heart disease

In addition to genetic influences factors which influence TAG levels are a high carbohydrate diet, overweight and excess alcohol and energy intake (Grundy & Denke, 1990; Assmann & Brewer, 1991; Carmena & Grundy, 1991). Although a high carbohydrate diet increase TAG levels this diet leads to the production of large buoyant VLDL particles which may be less atherogenic than dense VLDL particles (NIH Consensus Conference, 1993).

Although there are no clear indications that the lowering of elevated TAG levels will reduce the risk of CHD, the National Institutes of Health Consensus Development Conference suggested, in 1992, that lowering of TAG levels should form part of the therapy in those at increased risk of CHD (Adult Treatment Panel II, 1994). Overweight and excess energy intake influence TAG levels negatively and plasma TAG levels can be normalised by weight loss alone (NIH

Consensus Conference, 1993). Non-pharmacological measures should be the first step in the treatment of hyperTAG (>5.65 mmol/L) (NIH Consensus Conference, 1993).

2.1.4 Low density lipoprotein cholesterol and coronary heart disease

2.1.4.1 General information

In man, most of the cholesterol (65-70%) in serum is carried by LDL (Assmann, 1982). The LDLs arise from the catabolism of the TAG-rich VLDLs which are secreted by the liver. In the plasma, LPL which is present on the luminal surface of the capillary endothelium cells, is responsible for the hydrolysis of TAG from VLDL. VLDL is converted to IDL which is also called the VLDL remnant (Mahley, 1991; Ginsberg, 1990). Also see Figure 2.1. IDL is either taken up by the liver by interaction with the apo B/apo E receptor or further catabolised by hepatic lipase to LDL (Assmann, 1882). The main apolipoprotein in LDL is apo B-100 (Mahley, 1991) which originates from the metabolism of VLDL (Assman, 1982). Apo E and hepatic TAG lipase (HTGL) play important roles in the conversion of VLDL to IDL to LDL (Ginsberg, 1990). LDLs interact with LDL receptors on the hepatocyte and are taken up by the liver (Ginsberg, 1990). Apo B-100 and apo E are responsible for recognition of the lipoproteins by the LDL receptor (Mahley, 1991). LDL receptors are also found on human fibroblasts, smooth muscle cells of the arterial wall, and lymphocytes (Assmann, 1982). Between 40 and 60% of the LDL is taken up by the liver and the LDL receptor pathway is the main route (60-70%) of LDL-C uptake from the plasma (Ginsberg, 1990). LDL is probably also removed by a non-receptor pathway, namely scavenger receptors on macrophages and endothelial cells, because in homozygote patients with no LDL receptors roughly 15% of the LDL plasma pool is catabolised daily (Assmann, 1982; Kris-Etherton *et al.*, 1988).

Peroxidation of fatty acids in LDL particles in the plasma can take place and this seems to have an effect on the interaction of LDL with the cells. Cholesterol-laden macrophages or foam cells are formed as a result of the uptake of modified LDL by the scavenger receptors on the endothelial cells and macrophages (Ginsberg, 1990). Foam cells are a distinctive histological feature of atherosclerotic lesions (Grundy, 1991; Thompson, 1989).

2.1.4.2 Low density lipoprotein cholesterol and the risk of coronary heart disease

The association between high serum TC and CHD could mainly be ascribed to high LDL-C

levels (Grundy, 1991; Adult Treatment Panel II, 1994). Increased levels of both plasma LDL-C and LDL apo B are risk factors for the development of atherosclerosis and are associated with premature CHD (Ginsberg, 1990; Kannel *et al.*, 1979; Castelli *et al.*, 1986; Brunzell *et al*; 1984). A strong marker for the risk of developing premature CHD appears to be small TAG-enriched and relatively CE-depleted LDL particles (Ginsberg, 1990; Sniderman, Vu & Cianflone, 1991). LDL particle size, however, is also influenced by gender and dietary factors. Low saturated fat and low cholesterol intakes are associated with small dense LDL particles. LDL particle size may therefore not always be a good indicator of CHD risk in population studies (Campos *et al.*, 1992).

LDL-C levels are influenced by genetics and lifestyle. An elevated LDL-C level is a characteristic of familial hypercholesterolaemia which is known for the premature development of CHD (Assmann, 1982; Van Tol, 1986). LDL-C levels are also influenced by dietary cholesterol and saturated fat intake (Grundy & Denke, 1990).

2.1.4.3 Reference values for low density lipoprotein cholesterol

Reference values for LDL-C are given in Table 2.2. In persons without CHD and with less than two risk factors it is recommended to lower elevated LDL-C levels to less than 4.1 mmol/L, and in those with two or more risk factors without CHD, to less than 3.4 mmol/L (Adult Treatment Panel II, 1994). People with an LDL-C level above 4.14 mmol/L may require drug therapy (NIH Consensus Conference, 1993).

2.1.5 High density lipoprotein cholesterol and coronary heart disease

2.1.5.1 General information

Nascent HDL is produced by the liver and by the intestine and HDL is then fully developed in the plasma (Assmann, 1982). Unesterified cholesterol is accepted by the nascent HDL particles from extrahepatic cells. The enzyme lecithin:cholesterol acyltransferase (LCAT) esterifies the free cholesterol (Kris-Etherton *et al.*, 1988) by transferring a fatty acid from the ß-position of phosphatidylcholine to the 3-ß-hydroxy position of cholesterol to form CE (Assmann, 1982). Apo A-I is the cofactor used by LCAT. The esterified cholesterol moves to the core of the particle and as a result of the metabolism of chylomicrons and VLDL, surface components apo A, apo C and phospholipids are transferred to the HDL particle and mature HDL is formed

(Assmann, 1982; Kris-Etherton *et al.*, 1988; Tan *et al.*, 1993). Cholesterol ester transfer protein (CETP) is responsible for the transfer of CE in HDL_3 to apo B-containing lipoproteins, mainly VLDL (Mahley, 1991; Franceschini, Maderna & Sirtori, 1991). One mole of CE is exchanged for one mole of TAG between HDL and VLDL (Franceschini, Maderna & Sirtori, 1991). HDL_2 particles rich in TAG and CE are formed and as a result of hydrolysis by hepatic lipase the large HDL_2 particles are converted into small TAG and CE-poor HDL_3. HDL-C is probably involved in reverse cholesterol transport and carries the excess cholesterol from the peripheral tissues back to the liver for excretion (Mahley, 1991; Franceschini, Maderna & Sirtori, 1991).

2.1.5.2 High density lipoprotein cholesterol and the risk of coronary heart disease

It is generally agreed that a low HDL-C level is a risk factor for CHD and HDL therefore correlates inversely with the risk of CHD (Adult Treatment Panel II, 1994; Gordon, 1990). However, in non-industrialised countries in which the prevalence of CHD is low, low HDL-C as well as low TC levels are observed, but fat intake is also low (Gordon, 1990). In a study on 5 probands with very low HDL-C levels none had evidence of premature CHD but all had rapid catabolism of apo A-I and apo A-II (Rader *et al.*, 1993). There are also disease states such as familial LCAT deficiency or fish eye disease and Tangier disease which are characterised by severely reduced plasma HDL-C concentrations, but the risk of atherosclerosis may be normal or only moderately increased (Assmann, Von Eckhardstein & Funke, 1993).

There is an inverse association between HDL-C and TAG (NIH Consensus Conference, 1993). When the TAG-rich lipoproteins are present in excess this leads to TAG-enrichment of HDL through the action of CETP. The large HDLs (HDL_2) are transformed by hepatic lipase into smaller dense HDLs (HDL_3) which are cleared more rapidly from the blood and results in lower HDL levels (Shepherd & Krauss, 1991). CE is kept in the HDL when TAG-rich lipoprotein levels are low, because transfer of CEs from HDL into TAG-rich remnants are not allowed (Gotto *et al.*, 1991). This probably produces the anti-atherogenic effect of HDL.

High HDL, particularly HDL_2, is associated with a high LPL activity while low levels of HDL are associated with high hepatic lipase activity (Shepherd & Krauss, 1991). Atherosclerotic risk may be indicated by the ratio of LPL/hepatic lipase activity (Shepherd & Krauss, 1991). A high HDL_2 level indicates that TAG-rich lipoproteins are catabolised effectively and individuals with

high HDL_2 levels may thus be less prone to CHD (Gotto *et al.*, 1991). HDL_2 is a larger more lipid-rich and less dense particle while HDL_3 is a small lipid-poor and dense particle (Gotto *et al.*, 1991).

Significantly lower levels of HDL-C and HDL_3-C were reported in men with angiographically documented CHD than in those without it (Smuts *et al.*, 1994). Apolipoprotein A-I and apo A-II in the HDL and in HDL*3* were also significantly lower in the men with CHD than in those without it. HDL-C and apo A-I levels are higher in women than in men (Lyu *et al.*, 1993) which could explain the lower prevalence of CHD in premenopausal women than in men. It has been shown that with a 10% increase in apo A-I concentration the risk of CHD was 17% lower (Wald *et al.*, 1994).

2.1.5.3 High density lipoprotein cholesterol and the prevention of coronary heart disease

Lifestyle factors such as obesity, smoking and a sedentary lifestyle increase TAG levels and decrease HDL-C (NIH Consensus Conference, 1993). Changes in lifestyle such as regular exercise and weight loss may increase HDL-C by 10% to 20% (NIH Consensus Conference, 1993). Lower HDL-C and higher TAG levels are found in societies who consume a high carbohydrate diet compared to those who consume a Western diet. These lower HDL-C levels may, however, not be deleterious because LDL-C levels are also lower. In addition, high carbohydrate diets lead to large buoyant VLDL particles which are less atherogenic than dense VLDL particles (NIH Consensus Conference, 1993).

2.1.5.4 Reference values for high density lipoprotein cholesterol

Reference values for HDL-C are given in Table 2. There are indications that after adjustment to control for other risk factors an increase of 0.03 mmol/L in HDL-C results in a 2 to 3% decrease in CHD risk (NIH Consensus Conference, 1993; Adult Treatment Panel II, 1994).

Although ratios such as TC:HDL and LDL:HDL are used to indicate the risk of CHD it is recommended by the Adult Treatment Panel II of the National Cholesterol Education Program (NCEP, 1994) to focus on LDL-C and HDL-C separately as risk factors for CHD. Low HDL-C levels are usually not the main focus of treatment in the prevention of CHD. The primary aim is to lower LDL-C levels (Gordon, 1990). Nevertheless, a plethora of information

indicates that low HDL-C levels may be a risk factor for CHD. Therefore, community health means such as the prevention of obesity and inactivity should be the primary steps taken to treat low HDL-C levels.

2.1.6 Apolipoprotein B and coronary heart disease

2.1.6.1 General information

Apo B is synthesized in the intestine and the liver. It is a structural protein of chylomicrons, VLDL, IDL and LDL as well as lipoprotein(a) {Lp(a)} (Assmann, 1982). More than 90% of the apo B is carried in LDL in normolipidaemia (Assmann, 1982). In hyperTAG VLDL and chylomicrons contribute (20-50%) to total plasma apo B. If Lp(a) is elevated it also contributes to the total plasma apo B level (Assmann, 1982). Apo B is important for the secretion and transport of these lipoproteins (Assmann, 1982; Ginsberg, 1990). Two subtypes of apo B can be distinguished, namely apo B-48 and apo B-100 (Assmann, 1982). Apo B-48 is only made in the intestine and is necessary to collect and secrete the chylomicrons from the intestine (Ginsberg, 1990). The concentration of apo B-48 in the serum is unknown (Assman, 1982). Apo B-100 is a glycoprotein (Ginsberg, 1990) and originates in the ribosomes of the rough endoplasmic reticulum of the hepatic cell (Grundy, 1991; Mayes, 1983). Apo B-100 is necessary for the interaction of the LDL particle with the apo B/E receptor (Van Tol, 1986). It plays an important role in the plasma in the catabolism of the LDL particle and probably also of VLDL and IDL (Ginsberg, 1990). Apo B-100 is necessary for the secretion of VLDL (Van Tol, 1986). Many cases of hypercholesterolaemia could be ascribed to familial defective apo B-100 (Grundy, 1991).

Measurement of apo B, but not of LDL-C, gives an indication of the number of LDL particles in the plasma because the mass of protein per LDL particle is constant while the amount of cholesterol per particle can vary (Thompson, 1989; Sniderman, Vu & Cianflone, 1991). Every LDL particle only has one apo B (Grundy, 1991). An elevated LDL particle number can be recognized by the measurement of LDL-apo B in individuals with normal LDL-C and either normal or elevated TAG levels (Sniderman, Vu & Cianflone, 1991). The LDL-C level will be high for a given LDL-apo B level if the ratio of LDL-C:apo B is high (Grundy, 1991). In subjects with plasma TAG levels up to 5.6 mmol/L (500 mg/dL) a close correspondence between total apo B and LDL-apo B was found (Sniderman, Vu & Cianflone, 1991). Elevated

apo B seems to influence the association between hyperTAG and CHD. Only hyperTAG with an increased LDL particle number seems to be associated with increased risk for CHD (Sniderman *et al.*, 1982; Durrington *et al.*, 1986; Campeau *et al.*, 1984).

2.1.6.2 Apolipoprotein B and the risk of coronary heart disease

Whether apo B is a better predictor of CHD than LDL-C is not yet clear (Thompson, 1989). According to Sniderman, Vu & Cianflone (1991) evidence in the literature proves that the level of apo B correlates better with coronary disease risk than either TC or LDL-C. However, in a study on 440 men and women from a low CHD population (Tapei Chinese) and 428 men and women from a high CHD population (Framingham Americans), some of the twofold differences in age-adjusted CHD mortality between the two populations were explained by TC and especially by LDL-C but not by the apo A-I, apo B or Lp(a) values (Lyu *et al.*, 1993). It was shown in a prospective study on 21 520 men aged 35-64 years that the association between CHD and apo B decreased significantly with age (Wald *et al.*, 1994).

2.1.6.3 Reference values for apolipoprotein B

HyperapoB-lipoproteinaemia (hyperapoB) is characterised by an increased concentration of LDL-apo B (>120 mg/dL) and a normal concentration of LDL-C (<5 mmol/L) in the plasma (Thompson, 1989). HyperTAG is often also present. Since apo B is very hydrophobic it is difficult to analyze (Van Tol, 1986).

2.1.6.4 Apolipoprotein B and the prevention of coronary heart disease

Lifestyle plays a role in apo B levels and it was shown that body mass index (BMI) correlates positively with apo B (Lyu *et al.*, 1993). Apo B can be lowered by as much as 15% by lowering the total fat intake from 42 to 35% of total energy intake (Wald *et al.*, 1994). A prospective study on 21 520 men aged 35-64 years found that a 10% decrease in serum apo B, adjusted for apo A-I and apo(a), was associated with a decrease in CHD risk of 22% (Wald *et al.*, 1994).

2.2 The effect of diet on lipid and lipoprotein levels

2.2.1 Introduction

Diet plays an important role in the control of plasma lipid and lipoprotein levels. The amount and type of fat in the diet seem to be the major dietary factors which influence plasma lipid and lipoprotein levels. Researchers are generally in agreement that the SFAs, lauric, myristic and palmitic acids elevate plasma TC levels, while stearic acid does not. A plethora of information on the effect of dietary cholesterol on plasma lipoprotein levels indicates, however, that there is still controversy about its effect. Other dietary components such as carbohydrate, the type of fibre, alcohol and coffee intake also influence plasma lipid and lipoprotein levels.

The development of certain cancers may also be influenced by the diet. Fibre intake seems to have a protective effect while dietary fat is associated with breast and colo-rectal cancer (Bidoli *et al.*, 1992). A positive association between total fat intake and breast cancer and between saturated fat, adjusted for energy intake, and colorectal adenoma were reported (Magrath & Litvak, 1993; Giovannucci *et al.*, 1992). Although, especially dietary fat seems to play an important role in the development of cancer this issue will not be discussed below.

A discussion on the effect of dietary variables on plasma lipids and lipoproteins follows.

2.2.2 The effect of total fat intake on plasma lipids and lipoproteins

One of the major dietary recommendations for the lowering of plasma TC levels is to lower fat intake to less than 30% of energy (30%E) (Adult Treatment Panel II, 1994). Plasma concentrations of cholesterol can be lowered by either a very low fat diet (<10%E) or a low intake of SFAs (<6%E) (Morgan, Sinclair & O'Dea, 1993; Sanders *et al.*, 1994).

While a very low fat diet reduces LDL-C as well as HDL-C (Sanders *et al.*, 1994) it tends to increase TAG levels (Morgan, Sinclair & O'Dea, 1993; Sanders *et al.*, 1994; Grundy, 1986). A significant increase of TAG levels was observed with a decrease of total fat intake from 37 to 30%E (Howard *et al.*, 1995). Compared to a Western diet, a very low fat diet (<10% of energy) also resulted in a significant increase of serum TAG levels (Morgan, Sinclair &

O'Dea, 1993; Sanders *et al.*, 1994). TAG levels were also raised when a low fat (20%E) diet was compared with a high SFA diet (total fat = 40%E; SFA = 25%E) (Grundy, 1986).

On low fat diets the intake of carbohydrate usually increases. High carbohydrate diets induce the synthesis of VLDL-TAG (Melish *et al.*, 1980). When VLDL concentrations are elevated, HDL and VLDL exchange CE and TAG and this results in a relative depletion of cholesterol from the HDL particle (Blum *et al.*, 1977). This could explain the lowering of HDL-C levels found on a low fat diet (<10%E) (Morgan, Sinclair & O'Dea, 1993; Sanders *et al.*, 1994; Grundy, 1986). The LDL:HDL cholesterol ratio was found to be significantly higher on a low fat diet (20%E) compared to a high monounsaturated fatty acid (MUFA) diet (total fat = 40%E; MUFA = 28%E) (Grundy, 1986).

Compared to a low fat diet, a diet with a moderate fat content (30%E) containing either MUFA or PUFA and low in SFAs (<10%E) only lowered LDL-C (Sanders *et al.*, 1994). The addition of olive oil or safflower oil (20%E) to a very low fat diet (9%E) rich in lean beef, maintained the LDL-C lowering effect of the low fat diet (Morgan, Sinclair & O'Dea, 1993). HDL-C, however, increased with the addition of both oils to a very low fat diet, although the increase was not significant with olive oil (Morgan, Sinclair & O'Dea, 1993).

A moderate fat diet containing MUFA or PUFA did not increase TAG or lower HDL-C as did a very low fat diet (Morgan, Sinclair & O'Dea, 1993; Sanders *et al.*, 1994). A diet with a moderate fat content, therefore seems to be as effective as a very low fat diet in lowering LDL-C concentrations. An additional benefit of a moderate fat diet is the fact that it does not also lower HDL-C and increase TAG concentrations. It is important to note that in this moderate fat diet, the type of fat will determine cholesterol lowering effects.

2.2.3 The effect of the type of fat in the diet on plasma lipids and lipoproteins

2.2.3.1 General information

There are indications that in terms of the lipid profile the type of dietary fat may be of greater importance that the amount of fat in the diet. The characteristics of the particular dietary fat influences its effect on plasma lipids and lipoproteins. Factors which play a role are the number

of carbons in the fatty acid chain, the number of double bonds in the chain and the position of the double bond (Keys, Anderson & Grande, 1965a,b; Goodnight *et al.*, 1982). In addition, the *cis* and *trans* configuration of the double bond in the unsaturated fatty acids also influences the effect of the fatty acid on the lipid profile (Khosla & Hayes, 1996). Saturated fatty acids are generally associated with an increase in plasma TC and LDL-C but not all the SFAs seem to have this effect. *Trans* fatty acids are also associated with undesirable lipid responses (Judd *et al.*, 1994). MUFAs are regarded as neutral, while the PUFAs are associated with a more favourable lipid profile. The PUFAs are divided into two classes namely the omega-six (n-6) PUFAs and the n-3 PUFAs. Although both have a favourable effect on the lipoprotein profile their effect varies (Goodnight *et al.*, 1982).

The response of plasma lipids and lipoproteins to the type of dietary fat will also depend on what it replaces in the diet.

A discussion of studies which investigated the influence of the type of fat in the diet on plasma lipids and lipoproteins follows. The earlier studies of Keys, Anderson & Grande (1965b) and Hegsted *et al* (1965) indicated that not all the SFAs have a cholesterol elevating effect as well as more recent research which either confirmed or added new information to these finding are used in the discussion.

2.2.3.2 The effect of saturated fatty acids on plasma lipids and lipoproteins

(a) *Background*

Plasma cholesterol levels are more sensitive to the type of fatty acids than to the quantity of cholesterol in the diet (McNamara *et al.*, 1987). To lower TC and LDL-C concentrations a reduction in SFA intake and not total fat intake is required (Morgan, Sinclair & O'Dea, 1993). It is generally recommended to lower SFA intake to $<10\%$E for the general population and to $<7\%$E (the Step II diet) for those with hypercholesterolaemia (Adult Treatment Panel II, 1994).

There is little doubt that SFAs elevate plasma lipids and lipoproteins, although not all the SFAs have a cholesterol-elevating effect. A discussion of the effect of the different fatty acids on plasma lipids and lipoproteins follows.

(b) *The effect of the short and medium-chain saturated fatty acids*

Compared to the long-chain fatty acids which are transported via the lymph the medium chain fatty acids are absorbed much faster via the portal route (Du Pont, 1990). There are indications that SFAs with fewer than 12 carbons in the chain do not raise plasma cholesterol (Keys, Grande & Anderson, 1965b).

(c) *The effect of lauric, myristic, palmitic and stearic acids on plasma lipoproteins*

Saturated fatty acids responsible for the elevation of cholesterol levels are lauric, myristic and palmitic acids (Keys, Anderson & Grande, 1965b; Denke & Grundy, 1992; Zock, De Vries & Katan, 1994). A study by Hegsted *et al.* (1965) suggested that lauric acid does not have a cholesterol-elevating effect but this was not confirmed by others (Denke & Grundy, 1992). Lauric acid is less hypercholesterolaemic than palmitic acid (Denke & Grundy, 1992) and myristic acid is more hypercholesterolaemic than palmitic acid (Zock, De Vries & Katan, 1994). Zock, De Vries & Katan (1994) showed that myristic acid is one and a half times as cholesterol-raising as palmitic acid but part ("about one half") of the effect was due to an increase in HDL-C.

Compared to oleic acid, myristic acid as well as palmitic acid were found to raise LDL-C and apo B levels significantly (Zock, De Vries & Katan, 1994). A mixture of lauric and myristic acids, however, produced a higher serum cholesterol concentration than an isocaloric amount (5% of energy) of palmitic acid in normocholestrolaemic young men on a diet containing 200 mg cholesterol/day (Sundram, Hayes & Siru, 1994). In a study in which 40% of dietary energy came from fat the effect, of a synthetic myristic acid test fat on TC, LDL-C, and apo B concentrations, did not differ from that of palm oil (Tholstrup *et al.*, 1994b).

Denke & Grundy (1992) speculated that one of the reasons why lauric acid is less hypercholesterolaemic than palmitic acid could be related to the manner in which these fatty acids are delivered to the liver, i.e. via the portal route or by the chylomicrons. The medium chain TAGs which are not hypercholesterolaemic are mainly (95%) absorbed via the portal route (Swift *et al.*, 1990). Denke & Grundy (1992) quoted others who indicated that 25-30% of lauric acid is absorbed via the portal route compared to less than 5% of palmitic acid which is absorbed via this path.

Saturated fatty acids increase HDL-C when it replaces carbohydrate in the diet (Katan, Zock & Mensink, 1995). When 1% of energy from carbohydrate is replaced by 1% of energy from fatty acids, HDL-C seems to be the same on palmitic acid and oleic acid diets, while HDL-C is higher on myristic acid relative to the oleic acid (Katan, Zock & Mensink, 1995). Tholstrup *et al.* (1994b) also found a higher HDL-C concentration and a lower LDL:HDL cholesterol ratio with a high myristic acid compared to a high palmitic acid diet.

(d) ***The effect of stearic acid on plasma lipoproteins***

Stearic acid does not have a cholesterol-elevating effect (Keys, Anderson & Grande, 1965b; Hegsted *et al.*, 1965; Tholstrup *et al.*, 1994a; Bonanome & Grundy, 1988). Compared to diets enriched with either palmitic acid or myristic acid plus lauric acid, LDL-C levels were significantly lower on a diet enriched with stearic acid (Tholstrup *et al.*, 1994a).

Like oleic acid, stearic acid lowers LDL-C, although there were indications that it also lowers HDL-C relative to unsaturated fatty acids (Katan, Zock & Mensink, 1995). A lower HDL-C level was observed with a diet rich in stearic acid compared to a diet rich in palmitic acid (Tholstrup *et al.*, 1994a).

Malabsorption of stearic acid (Apgar *et al.*, 1987) or the conversion of stearic acid to oleic acid (Bonanome, Bennet & Grundy 1992) could explain why stearic acid does not increase plasma cholesterol levels. According to Emken (1992) the differences in the effect of stearic acid and palmitic acid on cholesterol levels cannot be explained by differences in absorption. Although they did not rule out the possibility of malabsorption, a study by Tholstrup *et al.* (1994a) also did not suggest that stearic acid was less well absorbed than palmitic, myristic or lauric acid. A study on rats indicated that the positional distribution of stearic acid on the TAG molecule may influence its absorption (Monsma & Ney, 1993). Stearic acid seems to be less well absorbed at the sn-1,3 position on the TAG molecule (Monsma & Ney, 1993).

Bonanome, Bennet & Grundy (1992) found that in mice stearic acid was rapidly converted to oleic acid and it is known that the latter does not raise plasma cholesterol. However, there are also indications from a study on humans that only small percentages of stearic acid (average 9,2%) were converted to oleic acid. The difference in effect of stearic acid and palmitic acid on cholesterol and LDL concentrations is therefore probably only partially explained by the

conversion of stearic acid to oleic acid (Emken, 1992).

2.2.3.3 *Trans* fatty acids and coronary heart disease

(a) *Background*

Epidemiological studies are limited in their ability to prove that a high intake of *trans* fatty acids promotes CHD (Allison, 1995). The study in eight European countries and Israel (EURAMIC) did not show a major overall effect of C18:1 *trans* fatty acids in adipose tissue, on the risk of acute myocardial infarction (Aro *et al.*, 1995). In agreement, a study by Roberts *et al.* (1995) also did not show a relation between *trans* isomers of oleic and linoleic acids combined and sudden cardiac death in men younger than 65 years of age. However, results from the Nurses' Health Study, a prospective cohort study, indicated that the consumption of partly hydrogenated vegetable oils may contribute to the occurrence of CHD (Willet *et al.*, 1993). *Trans* fatty acid intake was estimated by a semi-quantitative food frequency questionnaire in this study and it is known that there are limitations in estimating dietary intake reliably in free-living populations.

A cross-sectional study on patients who underwent coronary angiography did, however, also show that elaidic acid (18:1 omega 9t; $p = 0.0300$) and trans-10-octadecanoic acid (18:1 omega 8t; $p = 0.0434$), but not other *trans* fatty acids, in platelets were positively associated with the degree of coronary artery disease (Hodgson *et al.*, 1996)

(b) *Effect of* trans *fatty acids on plasma lipids and lipoproteins*

(i) Total cholesterol and low density lipoprotein cholesterol

Compared to an oleic acid enriched diet the TC elevating effect of *trans* fatty acids was found to be about half that of the SFAs lauric, myristic and palmitic acids (Mensink & Katan, 1990; Judd *et al.*, 1994). *Trans* fatty acids also raise LDL-C, although to a lesser extent than SFAs (Mensink & Katan, 1990; Zock & Katan, 1992). When oleic acid (*cis* C18:1) is converted to elaidic acid (*trans* C18:1 n-9) it loses its ability to increase hepatic receptor activity (Dietschy, 1995). This might explain the higher LDL-C levels on *trans* fatty acids than on oleic acid.

As a result of the LDL-C elevating effect and the HDL-C lowering effect found with a *trans* fatty acid enriched diet in the study of Mensink & Katan (1990) the ratio of LDL-C:HDL-C was higher on the *trans* fatty acid diet than on a diet high in SFAs.

(ii) High density lipoprotein cholesterol

Mensink & Katan (1990) observed an HDL-C lowering effect of *trans* fatty acids (≈ 11%E) compared to oleic acid or a SFA mixture of lauric, myristic and palmitic acids. The HDL-C lowering effect of *trans* fatty acids (Mensink & Katan, 1990) is not a consistent finding and it is not clear whether *trans* fatty acids have a dose-dependent effect on HDL-C (Denke, 1995). Judd *et al.* (1994) did not find a lowering of HDL-C with a *trans* fatty acid intake of 3%E which is regarded as the approximate intake of the American population. With a *trans* fatty acid intake of 6,6%E HDL-C was, however, significantly lower compared to oleic acid and SFA diets (Judd *et al.*, 1994).

(iii) Triacylglycerol

TAG levels were elevated to the same extent by *trans* fatty acids and SFAs in the study of Mensink & Katan (1990). In another study only the diet high (6,6%E) but not moderate (3,8%E) in *trans* fatty acid content, resulted in a significantly higher TAG level than the SFA enriched diet when compared to an oleic acid enriched diet (Judd *et al.*, 1994).

(c) *Effect of* trans *fatty acids on Lp(a) levels*

Trans fatty acids are one of the rare dietary factors found to increase Lp(a) (Katan, 1995). The Lp(a) elevating effect of *trans* fatty acids is, however, not a consistent finding (Denke, 1995).

2.2.3.4 The effect of monounsaturated fatty acids on plasma lipids and lipoproteins

(a) *Background*

The dietary recommendation for MUFA intake is an intake of up to 15%E (Adult Treatment Panel II, 1994).

Monounsaturated fatty acids do not seem to have an independent effect on plasma TC levels (Hegsted *et al.*, 1993). The effect of MUFA on cholesterol concentrations is neutral when compared to equal calories of simple carbohydrate, (Keys, Anderson & Grande, 1965a) but cholesterol-lowering when compared to SFAs (Katan, Zock & Mensink, 1995; Grundy, 1986). Keys, Anderson & Grande (1965a) showed that isocaloric exchange of oleic acid and erucic acid for simple carbohydrate had little or no effect on serum cholesterol levels. A meta-analysis of 27 trials also showed that the effect of MUFA relative to carbohydrate on LDL-C was not significantly different from zero (Mensink & Katan, 1992).

(b) ***Monounsaturated fatty acids versus low fat diet***

When the effects of liquid formula diets were tested, a diet high in MUFAs seemed to be as effective in lowering plasma TC as a low-fat high-carbohydrate diet (Grundy, 1986). Ten men consumed solid-food diets under strict supervision and the effect on plasma lipids of a diet high in SFAs and very high in cholesterol (High Sat + Chol) was compared with a diet high in MUFAs low in cholesterol (High Mono) as well as a diet low in fat and cholesterol and high in carbohydrate (Low Fat) (Grundy *et al.*, 1988b). This study confirmed the findings with the liquid formula diets and also showed the MUFA diet was as effective as the Low Fat diet in lowering plasma TC and LDL-C. Mensink & Katan (1987) also used strictly controlled solid food diets to compare the effect of a low-fat high-carbohydrate diet (total fat, 22.1%E) with a diet high in MUFAs (total fat, 40.6%E) on the plasma lipids of 12 men and 12 women They also found that both diets caused the same fall in plasma TC compared to a diet high in SFAs. In another study on eleven men in a metabolic ward, the effect of a diet enriched with olive oil (total fat, 38%E) was compared with a low fat diet (total fat, 28%E). Results showed that mean serum TC, LDL-C and apo B levels were significantly lower on the olive oil enriched diet than on the low fat diet (Baggio *et al.*, 1988).

A diet high in total fat (40%E) especially MUFAs do not seem to have the same HDL-C lowering effect as a low fat diet (20%E) high carbohydrate diet (Grundy, 1986, Mensink & Katan, 1987, Grundy *et al.*, 1988b). When olive oil was added to a very low fat diet, HDL-C increased, but not significantly (Morgan, Sinclair & O'Dea, 1993). However, others found a significant increase in HDL-C when olive oil (total fat = 30%E) was added to a low fat diet (<10%E) (Sanders *et al.*, 1994).

Studies in which the effect of a low-fat high-carbohydrate diet was compared with a high-fat high-MUFA diet showed lower TAG levels on the high MUFA diet (Grundy, 1986, Mensink & Katan, 1987). This difference between the effect of the two diets could be ascribed to an increase of carbohydrate on the low fat diet and will be discussed later.

(c) ***Monounsaturated fatty acids versus saturated fatty acids***

A high fat (40%E) liquid formula diet high in MUFA (28%E) resulted in a lower TC and LDL-C level than a liquid diet high in fat (40%E) and SFAs (18-25%E) (Grundy, 1986). This finding was confirmed by studies using strictly controlled solid food diets (Mensink & Katan,

1987; Grundy *et al.*, 1988b). The cholesterol-lowering effect observed with a diet high in MUFA is probably a result of an increased LDL receptor activity because SFAs in the diet are replaced and receptor activity is suppressed by SFAs (Grundy, 1987).

High density lipoprotein cholesterol levels and plasma TAG did not change when SFAs were replaced by MUFAs (Grundy, 1986; Mensink & Katan, 1987; Grundy *et al.*, 1988b).

(d) ***Monounsaturated fatty acids versus polyunsaturated fatty acids***

Oleic acid and linoleic acid seem to be equally effective in lowering plasma TC and LDL-C when they replace SFAs in the diet of normo-triacylglycerolaedemic subjects (Mattson & Grundy, 1985; Grundy, 1987; Mensink & Katan, 1989). Although a PUFA diet resulted in a greater decrease in TC and LDL-C than a MUFA diet in healthy men, the difference in the effect of the two diets was not significant (Bonanome *et al.*, 1992). In a study on hyperlipidaemic patients in a metabolic ward, Gustafsson, Vessby & Nydahl (1992) also did not find a significant difference between the effect of two diets, one high in MUFAs and the other high in PUFAs, on the TC and LDL-C levels.

In patients with normal TAG levels the substitution of PUFA for SFA lowered HDL-C significantly, but a MUFA diet did not have a significant effect (Mattson & Grundy, 1985). In a study on hyperlipidaemic patients, a PUFA diet decreased HDL-C more than a MUFA diet, 12% versus 8%, however, the difference between the effect of the two diets was not significant (Gustafsson, Vessby & Nydahl 1992). The advantage of MUFA over PUFA with respect to HDL-C was not confirmed by Dreon *et al.* (1990). In this study in which a fat-reduced (30%E total fat) solid food diet was used, PUFA did not lower HDL-C compared to MUFA.

There does not seem to be a difference in the effect of MUFAs versus PUFAs on TAG levels when SFAs in the diet are replaced (Mattson & Grundy, 1985; Dreon *et al.*, 1990). Howard *et al.* (1995), however, showed that the tendency of TAG levels to increase when total fat intake (37%E) is lowered seemed to be less if the moderate fat diet (30%E) contained a higher proportion of PUFA than MUFA (14%E vs 6%E).

2.2.3.5 The effect of polyunsaturated fatty acids on plasma lipids and lipoproteins

(a) *Background*

There are two families of the essential fatty acids the n-6 and the n-3 PUFAs. The parent fatty acid of the n-6 PUFAs is linoleic acid (C18:2n-6) while for the n-3 PUFAs it is linolenic acid. Vegetable oils such as sunflower, corn and safflower oils are rich sources of the n-6 PUFAs. Coconut oil, palm oil and cocoa butter are non-seed vegetable oils and they are not good sources of linoleic acid (Mahan & Arlin, 1992). In the Western diet the n-3 PUFAs mainly come from fish. Although the importance of the essential fatty acids in growth and development is recognised the role of the PUFAs, especially the n-6 PUFAs, in the prevention of CHD has been investigated for more than 40 years (Goodnight *et al.*, 1982). Interest in a possible protective effect of the n-3 PUFAs against the development of CHD has been stimulated by the low death rates from CHD in Greenland Eskimos and their high intake of the n-3 PUFAs (Bang & Dyerberg, 1972; Dyerberg, 1985).

A discussion of the influence of the PUFA content of the diet, the effect of the n-6 and n-3 PUFAs as well as the P/S ratio of the diet on plasma lipids and lipoproteins follows.

(b) *Total cholesterol and low density lipoprotein cholesterol*

The PUFAs seem to have a cholesterol-lowering effect over and above that of replacing SFAs in the diet (Mensink & Katan, 1992). Keys, Anderson & Grande (1965a) indicated that SFAs and PUFA have opposing effects and SFAs increase TC twice as much as PUFA lowers it. Although the replacement of SFAs by both MUFA and PUFA results in a better lipid profile, the latter seems to be slightly more favourable than MUFA (Mensink & Katan, 1992). Against the background of a diet containing 30%E from fat and 10%E from SFAs an increase in the intake of PUFAs (from 3%E to 14%E) at the expense of MUFAs, resulted in a decline in TC with less elevations in TAG and no effect on HDL-C (Howard *et al.*, 1995). There are also indications, however, that the exchange of MUFA for PUFA within the context of a lipid-lowering diet results in the same LDL-C and HDL-C concentrations (Lichtenstein *et al.*, 1993).

(c) *High density lipoprotein cholesterol*

Calculations from a meta-analysis of 27 studies showed that isocaloric replacement of SFAs in the diet by PUFAs would result in a statistically, but perhaps not biologically, significant decrease of HDL-C levels (Mensink & Katan, 1992). This meta-analysis also showed that

isocaloric replacement of carbohydrate by SFAs, PUFAs or MUFAs results in an increase in HDL-C with PUFA having the smallest effect of the three fats (Mensink & Katan, 1992). The HDL-C lowering effect of PUFA is not a consistent finding (Goodnight *et al.*, 1982). Increasing linoleic acid intake (from 3,7% to 12,7%) in a study on healthy men and women aged 18-65 years did not lower HDL-C (Valsta *et al.*, 1992). In agreement, increasing the PUFA intake from 7%E to 13%E in healthy normolipidaemic young men did not affect plasma HDL-C levels (Ginsberg *et al.*, 1994). There are indications that linoleic acid does not lower HDL-C if the intakes are less than 10-13%E (Mensink & Katan, 1989).

(d) ***Comparison of the effect of n-6 and n-3 PUFAs on plasma lipids and plasma lipoproteins***

Polyunsaturated fatty acids from vegetable oil (principally linoleic acid) lower plasma TC levels and LDL-C (Goodnight *et al.*, 1982). Although high intakes of fish oil (mean of 24 g n-3 PUFA) also lowered LDL-C (Illingworth *et al.*, 1984), a tendency of n-3 PUFAs to increase LDL-C in patients with hyperlipidaemia has been observed (Sullivan *et al.*, 1986; Demke *et al.*, 1988; Dart, Riemersma & Oliver, 1989; Kestin *et al.*, 1990a). In addition, the consumption of 15 g Maxepa per day raised LDL apo B in normolipidaemic as well as hyperTAG subjects significantly (Sullivan *et al.*, 1986).

The effect of n-6 and n-3 PUFAs on HDL-C varies (Goodnight *et al.*, 1982). In mildly hypercholesterolaemic subjects both linoleic acid and fish oil had little effect on HDL-C (Kestin *et al.*, 1990a). A daily intake of 1,4 g, 2,4 g, 4,1 g or 8,3 g of n-3 PUFAs did not change HDL-C levels (Bronsgeest-Schoute *et al.*, 1981) but HDL-C increased significantly on an eicosapentaenoic acid (EPA) intake of more than 3 g per day (Sanders & Roshanai, 1983; Saynor, Verel & Gillott, 1984). In normolipidaemic subjects a daily intake of 40 g Maxepa, however, decreased HDL-C significantly (Nestel, 1986). There are indications that HDL-C is more sensitive to docosahexaenoic acid (DHA) than to EPA (Herold & Kinsella, 1986).

The n-6 PUFAs do not seem to have a major effect on TAG levels although vegetable oil tends to lower TAG in hyperlipidaemic subjects (Goodnight *et al.*, 1982). The intake of vegetable oil rich in n-6 PUFA, however, decreased TAG levels in hyperTAG subjects (Phillipson *et al.*, 1985). Against the background of the NCEP Step II diet no significant effect on plasma TAG levels was observed in men and women with LDL-C concentrations >130 mg/dL, who

consumed corn oil rich in PUFAs (Lichtenstein *et al.*, 1993). PUFAs seem to have a more pronounced effect than MUFAs on serum total and VLDL-TAG, since both were significantly lower on a sunflower oil enriched than a rapeseed oil enriched diet (Valsta *et al.*, 1992).

A salient but consistent characteristic of n-3 PUFAs is to lower TAG levels in normo- as well as hyperlipidaemic subjects (Phillipson *et al.*, 1985; Sullivan *et al.*, 1986; Herold & Kinsella, 1986; Nestel, 1986; Dart, Riemersma & Oliver, 1989; Kestin *et al.*, 1990a).

(e) ***Polyunsaturated to saturated fatty acid ratio of the diet***

In healthy as well as hyperlipidaemic subjects TC and LDL-C concentrations decrease with an increase in the P/S ratio of the diet (Vessby *et al.*, 1980a; Blaton *et al.*, 1984; Jackson *et al.*, 1984; Fumeron *et al.*, 1991). Changing the P/S ratio of the diet without a concomitant change in the total fat content of the diet results in a less pronounced cholesterol-lowering effect in humans (Vessby *et al.*, 1980a). The mean reduction in plasma cholesterol levels of human subjects was found to be 5.5% on a 35%E from fat diet when the dietary P/S ratio was shifted from 0.3 to 1.8 (McNamara *et al.*, 1987). In the absence of cholesterol in the diet, an increase in the P/S ratio of the diet seems to be less effective in the lowering of plasma TC (Goodnight *et al.*, 1982).

In hyperlipoproteinaemic (HLP) subjects an increase in the dietary P/S ratio from 0.02 to 2.0 decreased TC and LDL-C in type IIb and IV subjects but it only decreased TC in type IIa patients (Vessby *et al.*, 1980a). A study on a group of hypercholesterolaemic subjects showed a decrease in plasma TC and LDL-C with a fourfold increase in the dietary P/S ratio from 0.46 to 1.59 (Blaton *et al.*, 1984).

A decrease in LDL-C with an increase in the P/S ratio of the diet from 0.4 to 1 or 2 has been accompanied by a decrease in HDL-C with the result that the ratio of HDL-C:TC or HDL-C:LDL-C did not change (Jackson *et al.*, 1984). No change in HDL-C has been observed in healthy subjects when the P/S ratio of the diet was increased (Blaton *et al.*, 1984; Fumeron *et al.*, 1991). Although an increase in the dietary P/S ratio from 0,2 to 1,1 did not change HDL-C it did lower HDL_2-C significantly (Fumeron *et al.*, 1991). This might cancel the beneficial effect of an increase in the P/S ratio of the diet on LDL-C (Fumeron *et al.*, 1991). Nevertheless, a moderate increase in the P/S ratio of the diet to 1 or to 2 does not seem to have

a detrimental effect on total HDL-C levels (Jackson *et al.*, 1984; Fumeron *et al.*, 1991).

In hypercholesterolaemic patients an increase in HDL-C has been observed with an increase in the P/S ratio of the diet from 0.46 to 1.59 (Blaton *et al.*, 1984). Compared to a dietary P/S ratio of 0.2 a diet with a P/S ratio of 2, however, lowered HDL-C concentrations significantly by 16% in HLP type IIa patients but not in HLP type IIb and type IV patients (Vessby *et al.*, 1980a).

Fumeron *et al.* (1991) observed a slight but significant decrease in TAG levels in healthy young males when the P/S ratio of the diet was increased from 0,2 to 1,1. Increasing the P/S ratio of the diet from 0,4 to 1, however, did not result in a difference in TAG levels in another study (Jackson *et al.*, 1984). In the latter study, however, a decrease in plasma TAG was observed with an increase in the P/S ratio of the diet from 1 to 2. VLDL-TAG decreased significantly in HPL type IV patients on a diet with a P/S ratio of 2 (Vessby *et al.*, 1980a).

2.2.4 The effect of dietary cholesterol on plasma lipids and lipoproteins

2.2.4.1 General information

Conflicting results on the effect of dietary cholesterol on plasma cholesterol have been reported. Some studies showed that dietary cholesterol increase plasma TC while others did not confirm this finding (Roberts *et al.*, 1981; Flaim *et al.*, 1981; Katan *et al.*, 1986; Edington *et al.*, 1987). A positive association between dietary cholesterol and the risk of death from CHD has been reported (Shekelle & Stamler, 1989). Although the quantity of cholesterol in the diet is not as important as the type of fat in the diet it does seem to influence plasma cholesterol levels (McNamara *et al.*, 1987; Grundy *et al.*, 1988a). There are, however, several factors which may influence the effect of dietary cholesterol on plasma lipids and lipoproteins. These factors and the influence of dietary cholesterol on the plasma lipids and lipoproteins will be discussed in this section.

2.2.4.2 Factors which may influence the effect of dietary cholesterol on plasma cholesterol

There are several factors which may influence the effect of dietary cholesterol on plasma cholesterol.

(a) *Absorption*

Adults absorb on average between 50 and 60% of dietary cholesterol, although values can range from 20 to 85% (McNamara, 1985). Many people can compensate for an increased intake of dietary cholesterol by decreased fractional absorption of cholesterol and/or decreased endogenous synthesis of cholesterol (McNamara *et al.*, 1987). There are, however, individuals in whom the feedback mechanism is ineffective and this would result in an increase in plasma TC levels with an increased intake of dietary cholesterol (McNamara *et al.*, 1987). Cholesterol absorption efficiency decreased significantly by 8% (SD 2) in men on a cholesterol intake of 878 mg/day but the absolute amount of cholesterol absorbed increased and was enough to increase LDL-C levels (Gylling & Miettinien, 1992). In the above-mentioned study the synthesis of cholesterol decreased but the decrease was not significant (Gylling & Miettinien, 1992). Subjects with high LDL-C levels seem to absorb more dietary cholesterol than those with low LDL-C levels (Grundy *et al.*, 1988a).

(b) ***Increased bile acid synthesis and cholesterol turnover***

Other factors which also influence the effect of a high intake of dietary cholesterol on plasma cholesterol are increased bile acid synthesis and increased cholesterol turnover rate (Gylling & Miettinien, 1992). The LDL receptor can be down-regulated in humans by a high intake of dietary cholesterol (Applebaum-Bowden *et al.*, 1984; Grundy *et al.*, 1988a) which will increase the hepatic content of cholesterol and result in suppression of LDL receptor activity (Grundy, 1987). An increase in bile acid synthesis was observed with a high cholesterol (878 mg/day) intake and this could contribute to a non-response to high cholesterol intake (Gylling & Miettinien, 1992).

(c) ***Apo E phenotypes***

The response to dietary cholesterol intake may be influenced by the apo E phenotypes. Those with the apo E4 and apo E3 allele respond to dietary cholesterol intake while those with the apo E2 alelle do not (Quintão, 1991; Gylling & Miettinien, 1992). Subjects with the apo E3 allele are less sensitive to dietary cholesterol than those with the apo E4 allele (Quintão, 1991; Gylling & Miettinen, 1992).

(d) ***Hypo- and hyperresponders***

Jacobs *et al.* (1983) suggested that nonresponse of TC to a change in diet is rare. Hypo- and

hyperresponse of TC to a change in dietary intake were reported (Jacobs *et al.*, 1983; Oh & Miller, 1985; Katan *et al.*, 1986). Oh & Miller (1985) defined hyperresponders as those in whom plasma TC values increased by 8% or more as a result of an increased intake of dietary cholesterol. Hyporesponders were defined as those in whom plasma TC did not rise or rose by less than 5%. Oh & Miller (1985) suggested that the hyperresponders in their study were less at risk for developing premature CHD than the hyporesponders. The mean plasma TC level of hyperresponders was significantly lower than those of hyporesponders at baseline. In addition, hyperresponders carried a greater proportion of cholesterol in HDL, and had lower LDL-C/HDL-C ratios than hyporesponders. The latter tended to carry a greater proportion of cholesterol in LDL (Oh & Miller, 1985). Hypercholesterolaemic subjects who were sensitive to a low fat diet (>10% fall in plasma cholesterol on a 25% fat and <200 mg/day cholesterol diet) were also found to be more responsive to dietary cholesterol intervention than those who were diet-insensitive (Clifton *et al.*, 1990). There are also indications of a positive correlation between baseline levels of HDL-C and the decrease in serum cholesterol levels after a decrease in dietary cholesterol intake (Beynen & Katan, 1985).

McNamara *et al.* (1987) found that the majority of subjects could compensate for an increase in cholesterol intake and they are of the opinion that a decrease in dietary cholesterol from 450 to <300 mg/day would not significantly reduce cholesterol levels in the general population. In this study 31% of subjects were, however, non-compensators i.e. they lacked precise feedback control of endogenous cholesterol synthesis (McNamara *et al.*, 1987). These subjects who cannot compensate for increased cholesterol intake may benefit from a reduction in cholesterol intake (McNamara *et al.*, 1987). It is estimated that by lowering dietary cholesterol intake from 500 to 300 mg/d on a 2 000 to 2 500 calorie diet, plasma TC will be lowered by about 8 to 10 mg/dL (Grundy *et al.*, 1988a).

2.2.4.3 Effect of dietary cholesterol on plasma lipids and lipoproteins

(a) *Total cholesterol*

Quintão (1991) eloquently summarised dietary cholesterol studies done between 1960 and 1990. Most of the studies done in metabolic wards, but also many studies done on free-living populations, showed an elevation of plasma TC levels with an increase in dietary cholesterol intake (Quintão, 1991; Gylling & Miettinen, 1992). The significant increase in plasma TC, as a result of cholesterol feeding, varied between 3 and 58% in the metabolic ward studies

(Quintão, 1991). There are also studies, the majority of which are studies on free-living populations, in which an increase in dietary cholesterol intake did not result in an increase in plasma cholesterol levels (Flynn *et al.*, 1979; Buzzard *et al.*, 1982; Edington *et al.*, 1987; Kestin *et al.*, 1989a; Quintão, 1991).

It is questioned whether plasma TC increases linearly or curvilinearly with an increase in cholesterol intake up to a level of 500 mg per day (Grundy *et al.*, 1988a). The studies reported by Quintão (1991) showed that plasma cholesterol increased by about 0.08 mmol/L (3 mg/dL) for every 100 mg of cholesterol added to the diet. Based on reported investigations Dr Fred Mattson estimated, however, that on average plasma TC rises by about 0.26 mmol/L (10 mg/dL) for each 100 mg of dietary cholesterol per 1000 calories (Grundy *et al.*, 1988a)

(b) ***Low density lipoprotein cholesterol***

LDL-C and LDL-apo B levels are raised by an increase in dietary cholesterol intake (Schonfeld *et al.*, 1982; Beynen & Katan, 1985; Grundy *et al.*, 1988a; Vorster *et al.*, 1987; Clifton *et al.*, 1990; Gylling & Miettinen, 1992). The type of fat in the diet seems to play a role in the effect of dietary cholesterol on LDL-C. While LDL-C levels increased significantly with a dietary cholesterol intake of 750 mg or 1500 mg on diets with a P/S ratio of 0.25 or 0.4 they did not increase on a dietary P/S ratio of 2.5 (Schonfeld *et al.*, 1982). McNamara *et al.* (1987) could, however, not find a difference in the response to dietary cholesterol intake between a PUFA (P/S $\approx$ 1.5) and SFA diet (P/S $\approx$ 0.3).

(c) ***High density lipoprotein cholesterol***

HDL-C levels also increase with a high cholesterol diet (Oh & Miller, 1985; Katan *et al.*, 1986; Grundy *et al.*, 1988a; Clifton *et al.*, 1990; Gylling & Miettinen, 1992) although this is not a consistent finding (Schonfeld *et al.*, 1982; Buzzard *et al.*, 1982; Sacks *et al.*, 1984; Flynn *et al.*, 1986; Edington *et al.*, 1987).

(d) ***Very low density lipoprotein cholesterol***

A high dietary cholesterol intake does not seem to influence VLDL-C significantly (Schonfeld *et al.*, 1982; Vorster *et al.*, 1987), however, in hyperresponders VLDL-C increased significantly as a result of an increase in dietary cholesterol (Oh & Miller, 1985).

(e) *Triacylglycerol*

TAG levels do not seem to be influenced by a high dietary cholesterol intake (Oh & Miller, 1985; Flynn *et al.*, 1986; Edington *et al.*, 1987; Gylling & Miettinen, 1992).

(f) *Chylomicron remnants*

There are indications that chylomicron remnants enriched with cholesterol accumulate in plasma when large quantities of cholesterol are consumed (Grundy *et al.*, 1988a). Chylomicron remnants are atherogenic (Slyper, 1992).

2.2.5 The effect of carbohydrate on plasma lipids and lipoproteins

2.2.5.1 General information

Carbohydrates vary from simple sugars to complex polymers (Mahan & Arlin, 1992). Carbohydrate is an important source of energy in the diet and at least half of the daily total energy intake should come from carbohydrate, especially complex carbohydrate. A decrease in total fat intake is usually accompanied by an increase in carbohydrate intake. The effect of carbohydrate on the plasma lipids and lipoproteins will determine to a large extent on what it replaces in the diet.

Dietary fibre (or non-starch polysaccharides plus lignin) is usually defined as the cell wall material from plant foods which are not digested by human enzymes. The dietary fibre hypothesis (reviewed by Vorster, 1994) that high fibre diets protect against the development of chronic diseases of lifestyle, has revolutionised the science of nutrition. Research on the physiological effect of fibre has, however, been hampered by a lack of agreement on definition, nomenclature, classification and methods of analysis (Cummings *et al.*, 1995). The effects of dietary fibre in the different parts of the digestive system and eventually on blood biochemistry and metabolism are influenced by the type of fibre (and therefore the type of food), its biochemical and physical composition, the amounts in the diet, and which foods or nutrients are replaced by a high fibre food or a fibre extract (Vorster, 1994).

In this section effects of total carbohydrate and sucrose will be discussed. The effects of some fibres on plasma lipids will also be briefly summarised without discussing other known

physiological effects or mechanisms of action.

2.2.5.2 The effect of total carbohydrate on plasma lipids and lipoproteins

(a) *Total cholesterol and low density lipoprotein cholesterol*

Carbohydrate seems to have approximately the same effect on plasma TC as MUFAs (Keys, Anderson & Grande, 1965a; Grundy *et al.*, 1988b). A low fat diet does not seem to be the only answer for lowering TC concentrations. The reduction of TC and LDL-C was similar on a 20%E fat with 65%E carbohydrate, or 40%E fat with 45%E carbohydrate, diet high in MUFAs and low in SFAs (Grundy *et al.*, 1988b).

(b) *High density lipoprotein cholesterol*

Low fat high carbohydrate diets seem to lower HDL-C. Replacing carbohydrate in the diet with fat, increases HDL-C. The higher the saturation of the fat the more pronounced is the effect (Katan, Zock & Mensink, 1995). Grundy *et al.* (1988b) found a significantly lower HDL-C concentration on solid food diets containing 20%E from fat compared to a 40%E diet high in either SFAs plus cholesterol or MUFA.

(c) *Triacylglycerol*

Short-term metabolic ward studies have shown that TAG levels are higher on low fat high carbohydrate diets than on high fat low carbohydrate diets (Baggio *et al.*, 1988; Nelson, Schmidt & Kelley, 1995). In 11 young male volunteers studied in a metabolic ward the mean TAG level was significantly lower on a MUFA enriched diet (38%E from fat) compared to a low fat diet (28%E from fat) (Baggio *et al.*, 1988). In agreement, another metabolic ward study on 11 male volunteers also showed significantly higher TAG levels on a low fat diet (22%E) than on a high fat diet (39%E) (Nelson, Schmidt & Kelley, 1995). Fasting TAGs decrease when fat replaces carbohydrates in the diet (Katan, Zock & Mensink, 1995). An increase in TAG levels in hypercholesterolaemic patients, however, was only observed with a carbohydrate intake of $\geq$60%E while no increase was observed in combined hyperlipidaemic subjects (Retzlaff *et al.*, 1995). In subjects who had relatively low TAG levels Grundy *et al.* (1988b) did not find a higher TAG level with a high carbohydrate (65%E) solid food diet compared to diets which contained 45%E from carbohydrate and was either high in SFAs plus cholesterol (900 mg) or high in MUFA.

Body weight tends to decrease on high carbohydrate low fat diets and could mask the TAG-elevating effect of a high carbohydrate intake (Retzlaf *et al.*, 1995).

The TAG elevating effect of a high carbohydrate intake does not seem to be transient because it was shown that boys from populations who consume more carbohydrate and less fat have higher TAG levels than boys from populations who consume less carbohydrate (West *et al.*, 1990). In addition, a two-year study on hypercholesterolaemic subjects also showed that after two years on a carbohydrate intake of ≥60%E TAG levels were significantly higher than at baseline (Retzlaf *et al.*, 1995).

Increasing carbohydrate intakes from 40 to ≥55%E has raised TAG levels in short-term studies (Frayn & Kingman, 1995). Increasing carbohydrate intake within the dietary recommendations, however, would probably not negatively influence the TAG levels of subjects with hypercholesterolaemia or combined hyperlipidaemia (Retzlaf *et al.*, 1995).

2.2.5.3 The effect of sucrose on plasma lipids and lipoproteins

(a) ***Total cholesterol***

According to information from studies summarised by Frayn & Kingman (1995) plasma TC concentrations either showed no change (sucrose = 11-65%E) or were raised (sucrose = 18-52%E) by a high sucrose intake. The effect of an increased sugar intake will probably depend on which component of the diet it replaces.

(b) ***High density lipoprotein cholesterol***

The influence of sucrose on HDL-C is inconclusive (Frayn & Kingman, 1995).

(c) ***Triacylglycerol***

The intake of very large amounts (>35%E) of sucrose increases plasma TAG but it has been shown that the effect depends on the sensitivity of the subjects, e.g. the elderly, men and sedentary subjects, and those with hyperinsulinaemia or established CHD may be more sensitive (Truswell, 1994; Frayn & Kingman, 1995).

Plasma TAG does not seem to be influenced by sucrose consumed in amounts typical of Western

diets (Frayn & Kingman, 1995). Scientists are of the opinion that if sucrose and other refined sugar intake is restricted to 10%E there is, in general, no danger of hyperTAG except in rare cases (Bruce & Asp, 1994).

2.2.5.4 The effect of dietary fibre on plasma lipids and lipoproteins

(a) *The effect of wheat bran on plasma lipids and lipoproteins*

Truswell (1995a) recently summarised studies in which the effects of dietary fibre on plasma lipids were investigated. Wheat fibre, which is an insoluble fibre, does not have a cholesterol-lowering effect. The study of Kestin *et al.* (1990b) on mildly hypercholesterolaemic men also showed that wheat bran and rice bran had little effect on plasma TC concentrations.

(b) *The effect of soluble fibre on plasma lipids and lipoproteins*

In contrast to insoluble fibre the soluble fibres, pectin, guar gum and to a lesser extent oat bran fibre, have cholesterol-lowering effects (Truswell, 1995a). Truswell (1995a) mentioned that in ten of the 19 studies he reviewed pectin decreased plasma TC. He also indicated that pectin lowers LDL-C but does not influence HDL-C and TAG levels. The effect of guar gum, another soluble fibre, is similar to that of pectin (Truswell, 1995a). Truswell (1995a) is of the opinion that the effect of oat bran has been "oversold" and that with intakes of as high as 90 g/day of oats it was difficult for researchers to find effects. The consumption of 95 g oat bran per day, resulted in a significant reduction in plasma TC and LDL-C concentrations in mildly hypercholesterolaemic (Kestin *et al.*, 1990b). A metabolic ward study on 12 hypercholesterolaemic men, however, showed significantly lower serum TC ($p<0.05$) and LDL-C ($p<0.025$) concentrations on a diet containing 25 g oats bran per day compared to the control diet containing corn flakes (Anderson *et al.*, 1990). Calculations from 22 trials indicated that oats would result in an unweighted mean reduction of 6% in plasma TC (Truswell, 1995a).

Fibre from beans also have a hypercholesterolaemic effect (Anderson *et al.*, 1984; Lo *et al.*, 1986). Supplementation of the diet with 100 g oat bran per day (dry weight) or 115 g dried beans (dry weight) had almost identical effects on serum TC, LDL-C and HDL-C concentrations of hypercholesterolaemic subjects (Anderson *et al.*, 1984). Total cholesterol decreased by 19% on the oat bran as well as the dried bean diets and LDL-C decreased by approximately 24%. Addition of 25 g soy fibre to the lipid-lowering diet of Type II-A hypercholesterolaemic subjects provided a significant additional reduction of plasma TC (13 mg/dL; $p<0.04$) and LDL-C

(12 mg/dL; $p<0.05$) to that achieved with a low-fat, low-cholesterol diet (Lo *et al.*, 1986)

Psyllium, another soluble fibre, also has a cholesterol-lowering effect (Bell *et al.*, 1989). A fibre intake of 40 g per day (mainly legumes, fruit and vegetables) has been shown to counteract the negative effect of a high carbohydrate diet (58%E) on TAG and HDL-C levels (Rivellese & Maffettone, 1995).

2.2.6 The effect of alcohol on plasma lipids and lipoproteins

A moderate intake of alcohol may protect against CHD but high intakes increase risk (Klatsky *et al.*, 1977; Marmot, 1991). One to two drinks of alcohol per day show a protective effect for CHD morbidity and mortality and are usually the number of drinks recommended in dietary guidelines (Marmot, 1991). A negative correlation between the intake of wine and CHD, but not with total mortality, has been found (Criqui & Ringel, 1994). There are, however, indications of an overall increased mortality risk for beer (Criqui & Ringel, 1994). Non-alcoholic components of red wine, such as the phenolic substances, may explain why the French, despite a high fat diet, have a low prevalence of CHD. This is also known as the "French paradox". Frankel *et al.* (1993) showed that polyphenols in red wine have potent antioxidant properties towards the oxidation of LDL-C in humans.

Although there is a positive association between alcohol intake and HDL-C which may be advantageous in terms of the prevention of CHD (Angelico *et al.*, 1982; Crouse & Grundy, 1984) there are also indications that alcohol increases TAG levels and the production of VLDL-TAG (Crouse & Grundy, 1984). The effect of alcohol on TAG levels depends to a large extent on underlying factors such as initial body weight and underlying hyperTAG (Crouse & Grundy, 1984). TAG levels seem to increase more readily in those who are obese.

Furthermore, there are strong indications that the consumption of three or more alcoholic drinks daily is a risk factor for hypertension (Klatsky *et al.*, 1977).

2.2.7 The effect of protein on plasma lipids and lipoproteins

In the Seven Countries Study a low correlation ($r = 0.14$) between the percentage of energy from protein and CHD incidence was found (Keys, 1970).

Forsythe *et al.* (1986) reviewed the effect of dietary protein on cholesterol and lipoprotein concentrations. It was shown that the replacement of mixed protein by soy protein in the diet reduces plasma TC in hypercholesterolaemic subjects, but results in only a small change in those with normal plasma TC concentrations. No significant difference in mean plasma TC and LDL-C concentrations of normolipidaemic young men was found when a diet in which 55%E was derived from beef protein was compared with a plant protein diet (Wiebe, Bruce & McDonald, 1984). In hyperlipidaemic individuals, by substituting soy-bean protein for beef protein in a low fat low cholesterol diet, showed that neither was superior to the other in modifying blood lipid levels (Holmes *et al.*, 1980). There are, however, also indications from other studies that soy protein diets will lower TC and LDL-C (Forsythe *et al.*, 1986).

2.2.8 The effect of coffee on plasma lipids and lipoproteins

There is a positive correlation between coffee consumption and serum TC (Curb *et al.*, 1986). Burr *et al.* (1995) found only a very small increase in serum TC with instant coffee. Caffeine does not seem to be responsible for the cholesterol-elevating effect of coffee (Superko *et al.*, 1991). In 108 men studied for two months plasma LDL-C and apo B lipoprotein concentrations were significantly higher on decaffeinated than on caffeinated coffee (Superko *et al.*, 1991).

The consumption of boiled coffee resulted in significantly higher levels of serum TC, LDL-C and apo B concentrations than filtered coffee (Aro, Teirilä & Gref, 1990). A lipid-rich fraction found in boiled coffee is probably responsible for the cholesterol-raising effect of boiled coffee (Zock *et al.*, 1990). Van Dusseldorp *et al.* (1991) found that the paper filters used to filter coffee held back the lipid fraction present in boiled coffee. This could explain why filtered coffee does not raise plasma TC levels.

2.3 Dietary guidelines for the prevention of coronary heart disease

2.3.1 Dietary guidelines for the general public

2.3.1.1 Historical background

The high prevalence of CHD in the Western world and the plethora of information on the relationship between diet and CHD set the scene for the formulation of dietary guidelines for

the prevention of CHD.

As early as the 19th century attention was given in Europe to dietary recommendations for appropriate energy and protein intake (Harper, 1987). These recommendations were mainly aimed at meeting the needs of the working force, providing nutritionally adequate food for the army, and preventing the negative outcomes of deprivation and starvation. Observations by Attwater in 1902 on the importance of choosing the correct food for health initiated the establishment of dietary guidelines in the United States of America (USA) (Anon, 1994). During 1929-1935, maintenance of health was added as an important element to the dietary recommendations, as the importance of several vitamins and minerals was then recognised (Harper, 1987).

Prior to 1977, the "Basic Four Food Groups" formed the basis of dietary advice in the USA with the aim of meeting micronutrient requirements. Interest in the relationship between nutrition and the development of chronic degenerative diseases of lifestyle resulted in a shift in emphasis from meeting micronutrient needs to changing the contribution of the macronutrients to total nutrient intake and reducing the intake of certain dietary constituents such as sodium and cholesterol (McNutt, 1980).

While the Recommended Dietary Allowances (RDAs) and Dietary Reference Values (DRVs) address the intake of enough of the essential nutrients, dietary goals or dietary guidelines are aimed at the prevention of degenerative diseases. RDAs are needed "now and every day" while the dietary guidelines are "targets to aim for" (Truswell, 1987). Dietary goals or guidelines are general recommendations and are the same for the whole population, while the RDAs and DRVs are broken down for different ages and sexes as well as for specific physiological states (Truswell, 1987). There is usually only one set of RDAs for a country but more than one set of dietary guidelines coexist in some countries (Truswell, 1987).

Interest in the USA in the relationship between diet and degenerative diseases already started in 1957 (McNutt, 1980) and the first diet-heart statement was published by the American Heart Association (AHA) in 1965 (Truswell, 1987). Eight dietary guidelines were released in 1968 by the AHA:" (1) reduce animal fat, (2) decrease saturated fats and increase polyunsaturated fats, (3) reduce cholesterol, (4) maintain ideal body weight, (5) apply dietary recommendations

early in life, (6) maintain the principles of good nutrition with the change in diet, (7) adhere to dietary recommendations and 8) make sound food habits a family affair." The increase of carbohydrate intake, to compensate for the reduction in energy from fat, and the lowering of sodium intake were included in the 1978 revision of the recommendations (McNutt, 1980).

In 1968 the first set of dietary goals was published for the Scandinavian countries (Truswell, 1987). The main ideas behind the development of these goals were the influence of mechanisation on physical activity and on energy intake. Both were lowered by mechanization and guidelines were needed to ensure that the nutritional requirements of the low energy diet were met. Fat intake had increased and this also had to be addressed in order to ensure nutritional adequacy on the low fat diet (Truswell, 1987). Another reason given for the goals was that a reduction in fat intake could lower energy intake (to prevent overweight) and the relationship between SFAs and atherosclerosis was also mentioned.

Already in 1975 Norway implemented a nutrition policy and four dietary goals were listed, namely: "reduce fat, increase starchy foods, reduce sugar and substitute polyunsaturated fats for saturated fats" (McNutt, 1980).

During the 1970s several countries, e.g. the USA, Australia, New Zealand, The Netherlands, the Federal Republic of Germany and Canada, made statements which concentrated on dietary modifications to reduce the risk of coronary heart disease (Truswell, 1987).

The first version of the Dietary Goals for the United States of America was published in February 1977 (American Dietetic Association, 1979) and this document was, according to Truswell (1987), written by a group of "politically interested activists with small knowledge of nutrition". The first USA Dietary Goals met with strong opposition but was approved by many of the principal USA researchers on diet and atherosclerosis (Truswell, 1987). The second edition of the Dietary Goals published in December 1977 was considerably revised (American Dietetic Association, 1979). The name changed from Dietary Goals in 1977 to Dietary Guidelines in 1980 and 1985. While the dietary goals were aimed at the community at large, i.e. "national target, expressed in terms of average consumption", the dietary guidelines were written for individuals, e.g. "What should you eat to stay healthy?" (Truswell, 1987). The Health and Human Services and the United States Department of Agriculture voluntarily issued

Dietary Guidelines for Americans in 1980, 1985 and 1990, but the latest issue in 1995 was the first to be issued by mandate. Dietary Guidelines for Americans will now be issued at least every five years (Report of the Dietary Guidelines Advisory Committee, 1995).

In 1979 the Australian dietary guidelines were adopted by the then Department of Health and in 1983 by the National Health and Medical Research Council. These guidelines were reviewed and the 1992 guidelines also included statements on calcium and iron (Commonwealth Department of Health, Housing and Community Services, 1993). The 1984 United Kingdom Committee on Medical Aspects of Food Policy (UK, COMA) report differed from previous recommendations published in the UK and was aimed at individuals rather than at the mean intake of the whole population (Cottrell, 1985). The latest issue of dietary guidelines for cardiovascular disease published in the UK in 1994 gives targets for the population older than five years (Choo, 1994).

In The Netherlands dietary guidelines, namely the "Richtlijnen Goede Voeding", were published by the "Voedingsraad" in 1986. In 1987 at the, Cholesterolconsensus, agreement was reached on the dietary prescriptions for people with elevated cholesterol levels (Anon, 1992).

The World Health Organisation (1990) published a set of population nutrient goals, giving lower and upper limits for population average intake. These, as well as other intermediate and ultimate nutrient goals for Europe (James, 1994) can form the basis for developing dietary guidelines on an individual or national level.

The first South African Dietary Recommendations for the prevention of CHD for the general public were compiled in June 1988 and published in December 1989 (Diet Consensus Panel, 1989). These guidelines were decided on at a consensus workshop attended by medical doctors, dietitians, nutritionists and other health professionals who were involved in the treatment of patients with CHD, or who were scientists involved in research on the relationship between diet and CHD. The formulation of these guidelines was based on national as well as international scientific information on the relationship between diet and CHD. The South Africa Department of Health published an updated set of dietary guidelines in 1992, but these were only qualitative and not quantitative dietary guidelines (The HMAC Subcommittee: Nutrition Services, 1992).

2.3.1.2 Dietary guidelines from different countries

Dietary guidelines from different countries are summarised in Table 2.3.

Some countries recommend the control of energy intake for the maintenance of body weight, but they do not all recommend eating a variety of food. All the countries give a quantitative guideline for total fat intake. In New Zealand, The Netherlands and the UK the recommendation for total fat intake is higher than the figure of ≤30%E generally recommended by the USA.

The majority of countries recommend a SFA intake of ≤10%E. Although a specific guideline for *trans* fatty acids is not given in the Canadian dietary guidelines it is recommended that current levels of intake should not be increased (Scientific Review Committee, 1990). New Zealand includes *trans* fatty acids in its recommendation for SFA intake. In general, a dietary cholesterol intake of ≤300 mg/day is recommended. Canada does not give a specific guideline for dietary cholesterol intake, but indicates that it is "not without importance" (Scientific Review Committee, 1990). A carbohydrate intake of ≥50%E is generally recommended. The South African dietary guidelines, however, do not include a recommendation for carbohydrate intake. The recommendation for sodium intake varies among countries but an intake of 3 g per day is generally recommended. The dietary guideline for salt intake is incorrect in the South African guidelines and should read 7 g, not 5 g, salt per day (Diet Consensus Panel, 1989).

2.3.1.3 Critical evaluation of dietary guidelines

(a) *Background*

Coronary heart disease and cancer are the major causes of death in Western societies. Dietary guidelines in these countries therefore focus mainly on the prevention of degenerative diseases ignoring the state of health of the rest of the nation (Harper, 1987). Degenerative diseases are often referred to as *lifestyle* diseases which, by implication, means a great deal of personal control and responsibility over these diseases (Harper, 1987). Harper (1987) questions this against the background of the influence of heredity and the ability of the individual to control the environment.

Table 2.3. Dietary guidelines of different countries

	America	Canada	Germany	New Zealand	The Netherlands	South Africa	Switzerland	United Kingdom
Reference	American Heart Association, 1993	Scientific Review Committee, 1990	Deutsche Gesellschaft fur Ernährung, 1991	Mann *et al.*, 1993	Van Dis & Beusekamp, 1987; Bengel & Van Zandvoort, 1992	Diet Consensus Panel, 1989	Arbeitsgruppe Lipide, 1992	Choo, 1994; Noticeboard, 1991*
Age	> 2yrs	> 2 yrs		> 2 yrs	> 3 yrs	>2 yrs		>5 yrs
Energy	✓	✓		✓	✓	✓		
Total protein		13-15%E					10-20%E	
Total fat	<30%E	≤30%E (39 g/4.2MJ)	30%E	33%E	30-35%E	≤30%E	≤30%E	35%E
SFAs†	<10%E	≤10%E (13 g/4.2MJ)	≤ one-third of total fat	SFAs + TFAs ≤12%E (8-12%E)	≤10%E	≤10%E	≤10%E	≤10%E
TFAs#				see SFAs		limited		≤2%E
MUFAs§			one-third of total fat	20%E (10-20%E)	MUFA:PUFA 1:2 to 1:1		≤10%E	
PUFAs¶	<10%E	n-6 3%E n-3 0.5%E n-6/n-3 4:1 to 10:1		±8%E (6-10%E)	see MUFA	≤10%E	≤10%E	<10%E* n-3 = 0.2 g/day
Cholesterol	≤300 mg/d	reduced	≤300 mg/d		≤33 mg/MJ max. 300 mg/day (140 mg/4.2 MJ)	≤300 mg/day	<300 mg/day	~245 mg/d

Table 2.3. (continued)

	America	Canada	Germany	New Zealand	The Nether-lands	South Africa	Switzerland	United Kingdom
Complex carbohydrate		55%E (165 g/ 4.2MJ)	>50%E	50-55%E	55%E		50-60%E	50%E
Fibre			30 g/day (3 g/MJ)	25-30 g/day (soluble fibre ¼ of total)	3 g/MJ	20-30 g/day	30-40 g/day	11-18 g/day* (NSP)**
Sugar			10%E		mono+disac-charides: 15-25%E)			10%E*
Alcohol		5%E (2 drinks/day		men 3 drinks/ day; women 2 drinks/day	2-3 drinks (not every day)	2-3 drinks/ day (10-20 g/day alcohol)		
Sodium		be reduced		≤7 g/day (120 mmoL/day)	max. 9 g salt	3 g/d (5 g/d NaCl)		6 g salt/day

*Reference: Notice board 1991; †Saturated fatty acids; *#Trans* fatty acids; §Monounsaturated fatty acids; ¶PPolyunsaturated fatty acids; **non-starch polysaccharide

In the setting of dietary goals, a distinction is often not made between recommended levels of intake for an individual and the recommended mean intake for the population (Pryer *et al.*, 1995). Fewer people will meet the dietary goals on an individual than on an average intake level (Pryer, Brunner & Marmot, 1994). The average intake of the population should meet the guidelines (James, 1994). Those with a moderate risk make out a large percentage of the population and will benefit from a population strategy (James, 1994). If the average intake of the population is too high a shift in the intake of the whole population is needed. Even the individual who is content with an intake which is slightly too high needs to make adjustments (James, 1994). An individual may not be concerned about slightly elevated risk factors for CHD, but this may have major implications for the government in the provision of expensive care facilities for those with myocardial infarction, stroke, cancer and diabetes mellitus (James, 1994). A population strategy in the formulation of dietary guidelines rather than only concentrating on those with a high-risk may be of greater benefit to a country.

Although scientific evidence is at the root of dietary guidelines the numerical figures reflect some pragmatism (Dalen, 1991). An evaluation of the quantitative dietary guidelines for the prevention of the diseases of lifestyle follows.

(b) ***Evaluation of quantitative dietary guidelines***

(i) **Total fat intake should be ≤30%E (or 30 to 35%E)**

Perhaps the recommendation to restrict total fat intake to 30%E or less needs to be based on a stronger scientific basis than what presently exists (Brown, 1990).

Populations with a total fat consumption of less than 25%E have a low prevalence of CHD but so do those in Mediterranean countries where total fat intake is approximately 40%E (Keys, 1970). A very low fat diet, however, tends to lower HDL-C levels and increase TAG levels (Sanders *et al.*, 1994). A low fat diet may also decrease the palatability of the diet and may also provide less satiety than a higher fat intake.

The lowering of SFA intake remains the most important dietary recommendation for the lowering of TC and LDL-C and in populations following a Western diet this may best be achieved by the lowering of total fat intake. An intake of 30%E seems to be a good compromise between the advantages of a low fat diet and the Mediterranean diet containing

approximately 40%E from fat (Grundy, 1987). Most of the fat in the Mediterranean diet, however, comes from olive oil which is rich in MUFAs.

A serious shortcoming of quantitative guidelines for fat intake is the absence of a guideline for the lower limit of fat intake. The exception is the World Health Organisation (WHO) guidelines which also included a lower limit for fat intake (James, 1994; WHO & FAO Joint Consultation, 1995). In most adults at least 15% of energy should come from fat while women of productive age need at least 20%E from fat (WHO & FAO Joint Consultation, 1995). Children, at least until the age of 2 years, need 30-40%E from fat (WHO & FAO Joint Consultation, 1995). In China the average fat intake is about 14% with only a small portion of the population being overweight and about 13% having the first degree of chronic energy deficiency (James, 1994). The recommendation for the lower limit of 15%E for total fat intake, therefore seems to have epidemiological backing.

(ii) Saturated fatty acid intake should be ≤10%E

Results from the Seven Countries Study serve as a reliable guide for choosing the quantitative guideline for SFAs. The prevalence of CHD was lower in those countries where SFA intake was 10%E or less, than in those countries where approximately 20%E came from SFAs (Keys, 1970).

Although there is scientific evidence to restrict SFA intake to below 10%E, the level below 10%E which would be best, is not clear (Dalen, 1991). In addition, no indication is given whether a distinction should be made between those SFAs that increase TC and LDL-C and those that do not.

(iii) Dietary recommendations and *trans* fatty acids

The American Society of Clinical Nutrition and the American Institute of Nutrition Task Force on *Trans* Fatty Acids could not conclude from the evidence available to date that the intake of *trans* fatty acids is a risk factor for CHD (ASCN/AIN Task Force on *Trans* Fatty Acids, 1996). There are, however, others who did show a positive relationship between *trans* fatty acids and the degree of coronary artery disease in patients who underwent coronary angiography (Hodgson *et al.*, 1996) Regarding fatty acids, the lowering of SFAs remains the most important dietary recommendation, for the lowering of total and LDL-C (ASCN/AIN Task Force on *Trans* Fatty

Acids, 1996). However, for nutrition education purposes *trans* fatty acids should be regarded as a SFA (Katan, 1995).

(iv) Restrict dietary cholesterol intake to less than 300 mg per day

The body synthesises its own cholesterol and there is therefore no real need to consume dietary cholesterol (Brown, 1990). Foods containing cholesterol, such as animal products, however, make the diet more palatable.

There is still controversy about the effect of dietary cholesterol on plasma cholesterol. There is also considerable variation in the response of individuals to the intake of dietary cholesterol (Katan *et al.*, 1986). The effect of dietary cholesterol on plasma cholesterol is not linear and most changes are observed between an intake of 0 to 200 mg per 4.2 MJ per day (Brown, 1990). Nevertheless, dietary cholesterol does increase TC and LDL-C in well controlled studies and it may be atherogenic (American Heart Association, 1993). Therefore, even countries such as Canada that do not give a quantitative dietary guideline for cholesterol intake indicated that its effect on plasma cholesterol should not be ignored (Scientific Review Committee, 1990).

The guideline to lower dietary cholesterol intake to less than 300 mg per day does not take into account that the recommendation applies to active adult men (Brown, 1990). Women and small, inactive men will need less cholesterol to achieve the same degree of cholesterol lowering (Brown, 1990). Perhaps the guideline for dietary cholesterol intake should be in milligrams cholesterol per 4.2 MJ (1 000 kcal) per day rather than a single figure which ignores energy needs.

(v) Polyunsaturated fatty acids should provide up to 10%E

Since no free-living populations have consumed more than 10%E from PUFA over a prolonged period of time it is not regarded as safe to consume more than 10%E from PUFA (Brown, 1990; American Heart Association, 1993). There has been concern that a high intake of PUFA may promote cancer or lead to the oxidative modification of lipoproteins which probably play a role in atherosclerosis (American Heart Association, 1993).

The recommendation for PUFA intake refers to n-6 and n-3 PUFA intake. Although much attention is given to the role of n-3 PUFAs in the prevention of CHD in scientific literature,

very few give a specific recommendation for n-3 PUFA intake. Only Canada and the UK presently have a quantitative dietary guideline for the consumption of n-3 PUFAs (Table 2.3). Brown (1990) is of the opinion that 2 to 3%E from n-3 PUFAs may have a potential benefit. Canada also recommends an n-6/n-3 ratio of 4:1 to 10:1. The former might be difficult to achieve within the framework of a Western diet. Those who cannot meet the recommendation of a n-6/n-3 ratio of 4:1 or 10:1 are advised to eat more fish (WHO and FAO Joint Consultation, 1995).

Countries such as Israel and Finland now have PUFA intakes of 9%E and 10%E respectively, but in time the health implications of this level of intake will have to be shown (Brown, 1990).

(vi) Monounsaturated fat should provide about 15%E

In the diet MUFAS should probably be the predominant fatty acid. Like carbohydrate, MUFA lowers TC and LDL-C when it replaces SFAs in the diet but without lowering HDL-C (Grundy, 1987). The recommendation that MUFAs should provide about 15%E on a 30%E diet, and even 20%E should the diet provide 35%E, seems to be a realistic recommendation (Grundy, 1989).

(vii) Increase carbohydrate intake to ≥55%E

The recommendation to decrease total fat intake implies that the intake of carbohydrate or protein should be increased. In general the intake of protein is adequate in Western societies. Therefore, carbohydrate rather than protein should replace total fat in the diet.

A carbohydrate intake of ≥55%E may raise TAG levels especially in the short term (Frayn & Kingman, 1995). An increase in fibre intake may, however, counteract the negative effect of a high carbohydrate diet on TAG levels (Rivellese & Maffetone, 1995). It is not always taken into account that the recommendation to lower sugar intake also forms part of the recommendation for carbohydrate intake. Few countries have a quantitative dietary guideline for sugar intake (Table 2.3). A high intake of sucrose may affect plasma cholesterol concentrations negatively but there is considerable variation in its effect (Frayn & Kingman, 1995). Nevertheless, it is generally agreed that if sugar should supply 10% or less of energy there would be no danger of hyperTAG (Bruce & Asp, 1994).

(viii) Fibre

It is generally accepted that insoluble dietary fibre is important for gastrointestinal function, especially the prevention of constipation, and that soluble fibre lowers TC. Dietary guidelines therefore encourage the consumption of complex carbohydrate and fibre-rich foods such as fruit and vegetables, oat bran and legumes. It is, however, also acknowledged that an unrealistic high intake of insoluble and soluble fibre may have gastrointestinal side-effects and may interfere with the absorption of nutrients such as calcium (Adult Treatment Panel II, 1994).

At this stage there seems to be enough scientific evidence for a qualitative but not for a quantitative recommendation for dietary fibre (Brown, 1990; Scientific Review Committee, 1990).

Nevertheless, some countries have a quantitative dietary guideline for fibre and it varies between 25 g and 40 g per day (Table 2.3). In the UK, a separate guideline for non-starch polysaccharides (NSP) is also given (James, 1994).

(ix) Protein

The recommendation for protein varies between 10% and 20%E and would be enough to meet the recommendation of 0.8 g/kg on most energy intake levels (Subcommittee on the Tenth Edition of the RDAs, 1989). If only 10%E to 15%E of protein is recommended the RDA for protein may, however, prove to be difficult to meet on slimming diets providing 5 000 kJ or less energy. This needs to be addressed in the formulation of dietary guidelines for individuals.

In South Africa those who follow a Western diet consume approximately 15%E of their energy as protein and no change in intake is recommended (Langenhoven *et al.*, 1988b).

(x) Alcohol

The recommendation of one to two alcoholic drinks per day could be justified from the literature although higher intakes seem to be detrimental to health (Klatsky *et al.*, 1977, Scientific Review Committee, 1990; Rimm *et al.*, 1991).

The dietary guideline for alcohol intake serves as a guideline for those who consume alcohol on a regular basis. It does, however, not intend to encourage those who do not drink to consume

alcohol regularly. There seems to be no safe level of alcohol intake and dietary guidelines should advise pregnant women not to consume alcoholic drinks (Moushmoush & Abi-Mansour, 1991).

(xi) Sodium intake

The recommendation for sodium/salt intake differs among countries (Table 2.3).

It is accepted that sodium (salt) plays a role in hypertension (American Heart Association, 1988; American Heart Association, 1993; Adult Treatment Panel II, 1994). A restriction of salt intake may lower blood pressure levels (Alderman, 1994) but there is also genetic variation in the response to salt intake (American Heart Association, 1993). Weinberger *et al.* (1986) showed that significantly more hypertensive than normotensive subjects were sodium-sensitive. Identification of salt-sensitive people at a population level is, however, impractical (American Heart Association, 1993). It is argued that the restriction of salt intake for the general population may not be necessary for a large part of the population (American Heart Association, 1993). A shift in emphasis from the relationship between salt intake and hypertension to dietary advice has taken place in the latest issue of the USA Dietary Guidelines (Dietary Guidelines Advisory Committee, 1995). It emphasises that the diet as a whole should be low in sodium (Kennedy *et al.*, 1996). Salt is an essential nutrient but it is generally overconsumed. The link between sodium intake and hypertension is still being researched.

The AHA believes that the recommendation to restrict sodium intake to 3 g per day is supported by epidemiological evidence (American Heart Association, 1988). According to the rationale for the Canadian dietary guidelines there is not enough evidence to give a quantitative guideline for sodium (Scientific Review Committee, 1990). It is nevertheless recommended that the current level of sodium intake in Canada should be reduced.

The minimum requirement for sodium has not been unequivocally determined. Some sources give it as about 80 mg per day for an adult man weighing 70 kg (Scientific Review Committee, 1990) while other sources say 500 mg/day of sodium is the amount necessary for health (Adult Treatment Panel II, 1994). This is much lower than the estimated intake of sodium in Western countries which varies between 2.3 g and 6.7 g (Scientific Review Committee, 1990). The recommendation to lower sodium intake to 3 mg (5 g sodium chloride) per day therefore does

not seem to be harmful. In the NCEP (1994) a sodium intake of 2400 mg per day is recommended. This is also the level of intake recommended for the general public by the National High Blood Pressure Education Program in America (Adult Treatment Panel II, 1994).

Sodium chloride may be more harmful than sodium alone or in combination with other anions (Scientific Review Committee, 1990; American Heart Association, 1993). Attention should therefore specifically be given to the intake of sodium chloride and this should be reflected in dietary recommendations or guidelines for salt intake.

(c) *Dietary compliance*

Studies on populations as well as on selected study groups showed that compliance with dietary guidelines is not good (Black, Ravenscroft & Sims, 1984; Wolmarans *et al.*, 1988; Pryer *et al.*, 1995). A study on white South Africans showed that only 23% of men and 19% of women met the guideline for total fat intake ($\leq$30%E) while 17% of the men and women met the saturated fat guideline of $\leq$10%E (Wolmarans *et al.*, 1988). Few British men (5.2%) and women (7.4%) appeared to meet all three COMA guidelines for fat (Pryer *et al.*, 1995). Black, Ravenscroft & Sims (1984) found that only 7% of the dietitians they studied met the quantitative guideline for fibre intake while only 10% met the fat recommendation. Another study on British dietitians showed that within the first week of the study only 7% of the participants came within 10% of achieving the dietary goals. The mean intake of the group, however, met all the National Advisory Committee on Nutrition Education (NACNE) short-term goals except, for fat (Cole-Hamilton *et al.*, 1986).

Statistical analysis of the data may contribute to the findings of a low compliance rate with dietary guidelines. Determining how many individuals met a specific dietary goal rather than looking at how many had an average intake which met the target could have contributed to the low figures reported for dietary compliance (Pryer *et al.*, 1995). It was also shown in a group of highly motivated participants, namely dietitians, that they met the guidelines as a group, but it was especially difficult for those with high intakes to achieve the proposed dietary guidelines individually (Cole-Hamilton *et al.*, 1986).

There are several reasons why the population finds it difficult to meet dietary goals (Goldberg, 1992). Dietary guidelines are often confusing because the message is not clear. Some

guidelines, e.g. to limit the intake of eggs in order to decrease total cholesterol intake, are easy to follow but do not address the problem of a too high intake of saturated fatty acids. The media contributes to the confusion by the overhasty publication of results from new studies, especially those with a provocative nature (Goldberg, 1992). In addition, the need to adapt dietary guidelines as new scientific evidence becomes available, may be regarded by the public as a sign that scientists are indecisive. Very often there is a perception among the public that it is too expensive to follow a diet that will meet the dietary goals. The sometimes pragmatic way in which certain dietary goals were set could have resulted in unrealistic dietary goals which are difficult to achieve (Gibney, 1990). All these factors may contribute to the low percentage of people who comply with dietary recommendations.

(d) ***Conclusion***

Dietary recommendations are based on the belief that the modifications of risk factors will lower the prevalence of CHD. There seems to be enough evidence for defining quantitative dietary guidelines for some nutrients but for others only qualitative guidelines seem to be prudent.

Although quantitative dietary guidelines are a valuable tool for the nutrition scientist, it often confuses the general public. Quantitative dietary guidelines should be translated into easy to understand qualitative dietary guidelines for the general public. To improve compliance and sustainability, guidelines should also be practical, culture sensitive, affordable and lead to palatable diets. Specific quantitative and qualitative dietary guidelines should be formulated for those who are at increased risk of CHD as a result of hyperlipidaemia.

2.3.2 Dietary treatment for patients with hyperlipidaemia

2.3.2.1 Hypercholesterolaemia

Dietary intervention is the first step in the treatment of hypercholesterolaemia. In the USA the Step I and Step II diets are prescribed for those with elevated TC concentrations (Adult Treatment Panel II, 1994). The Step I diet is the diet also prescribed for the general public and, should dietary treatment on this Step I diet fail, the Step II diet is prescribed. On the Step II diet 7%E from SFA and <200 mg/day dietary cholesterol are prescribed in contrast to 8%E-10%E SFA and <300 mg/day dietary cholesterol on the Step I diet. The remainder of the dietary prescription on the Step I and Step II diets is the same, e.g. total fat ≤ 30%E, PUFAs

up to 10%E, MUFAs up to 15%E, carbohydrates ≥55%E, protein approximately 15%E, and is recommended to achieve and maintain desirable weight (Adult Treatment Panel II, 1994). The main aim of dietary treatment is to lower the LDL-C concentration to below 4.1 mmol/L (Adult Treatment Panel II, 1994). It is recommended that patients with hypercholesterolaemia but without CHD receive dietary treatment for at least six months before drug treatment is introduced (Schaefer, 1993). The services of a dietitian are usually required for those with hypercholesterolaemia in order to achieve the maximum effect of dietary treatment (Adult Treatment Panel II, 1994). In those with hypercholesterolaemia and CHD, drug treatment could be introduced earlier. In free-living populations a reduction in LDL-C concentrations of 5% on the Step II diet has been shown but under metabolic ward conditions reductions of 15-20% have been observed (Schaefer, 1993).

2.3.2.2 Hypertriacylglyceroledaemia

Patients with severe chylomicronaemia (HLP type I) may need very low fat diets (10 to 20%E) to prevent pancreatitis. Those with borderline hyperTAG could follow a diet providing 30%E from fat, 10%E from SFAs, and less than 300 mg cholesterol per day (Consensus Conference, 1984). Further restriction of fat may be needed should the plasma TAG levels not normalise (Consensus Conference, 1984).

2.3.2.3 South African dietary guidelines for hyperlipidaemia

Separate dietary guidelines for those with hypercholesterolaemia have not been formulated in South Africa. Against the background of the high prevalence of hypercholesterolaemia in parts of the South African population this lack of specific dietary recommendations for those with hypercholesterolaemia needs to be addressed when the dietary guidelines for South Africa are reviewed in future. The Medical Research Council (MRC) in collaboration with the Heart Foundation of Southern Africa have, however, designed two diets. The diets are the Step 1 diet for those with moderately raised TC and TAG concentrations providing 30%E from fat, and the Step 2 diet for those with markedly raised TC and TAG concentrations providing 25%E (Medical Research Council & Heart Foundation of Southern Africa, 1993). The Step 1 diet is also the diet prescribed for the general South African public. To meet the recommendation for total and SFA, intakes of lean red meat, chicken without skin, fish, low fat (Step 1 diet) or skimmed (Step 2 diet) milk and milk products and less fat in food preparation and on bread are recommended. In order to control dietary cholesterol intake three eggs per week are allowed

on the Step 1 diet and two eggs on the Step 2 diet.

2.4 Red meat in the diet

2.4.1 Introduction

Red meat forms a very important part of the diet of most South Africans who follow a Western diet. In 1992 the *per capita* consumption of red meat was 24.19 kg for the estimated total population of 31 857 000 (personal communications, South African Meat Board).

There is, however, much controversy surrounding the inclusion of red meat in the diet, especially in the diets of those with hyperlipidaemia. Despite its good qualities, red meat is associated with the development of CHD, and cancer of the colon and rectum (Bidoli *et al.*, 1991; Giovannucci *et al.*, 1992). Many consumers and representatives of the health fraternity are therefore of the opinion that red meat should be excluded from the diet or be severely restricted. The main reason for this is because red meat has been implicated as a major source of fat, especially SFA, and cholesterol in the diet.

2.4.2 Nutrient composition of red meat

Red meat possesses several good qualities. It is a good source of protein with a high biological value. The amino acid composition of red meat does not differ significantly from that of white meat, fish and poultry. Red meat supplies all the essential amino acids (Kruger *et al.*, 1992). Red meat is an important source of vitamin B_{12}, and beef is a better source of vitamin B_{12} than pork and chicken breast (Mahan & Arlin, 1992). Red meat is also a very important source of zinc which is especially important for normal growth in children. Lean beef is a good source of folic acid and an important source of riboflavin (Mahan & Arlin, 1992). In the British and American diets approximately half of the total intake of niacin and one-quarter of riboflavin comes from meat (Passmore & Eastwood, 1986). Red meat is also an important source of vitamin B_6, thiamin (especially pork) and pantothenic acid (Mahan & Arlin, 1992; Langenhoven *et al.*, 1991).

Animal products are the only source of haem-iron in the diet and 50-60% of the iron in beef, mutton and chicken is in the form of haem (Monsen *et al.*, 1978). Beef, however, is a better source of haem-iron than chicken breast (Hazell & Southgate, 1985) and also the dark meat of

chicken because its total iron content is higher (Langenhoven *et al.*, 1991). Approximately 30-40% of the iron in liver, pork and fish is in the form of haem-iron (Monsen *et al.*, 1978).

A high percentage of fat from red meat is saturated. In beef 50% of the fat is saturated, in mutton 47%, and in pork 39%, while the SFA content of fat from white meat is lower, ie 30% in chicken and 28% in cod liver oil (fish oil) (Langenhoven *et al.*, 1991). Myristic, palmitic and stearic acids are the main SFAs present in red meat (Kruger *et al.*, 1992). MUFAs also form an appreciable part of the fat in beef (42%), mutton (41%), pork (45%) and chicken (45%). The PUFA content of beef fat is only 4%, while it is approximately 8% in mutton fat, 11% in lard and 21% in chicken fat (Langenhoven *et al.*, 1991). The MUFA content of cod liver oil is 26% while its PUFA content is 39% (Langenhoven *et al.*, 1991).

There is not a significant difference between the cholesterol content of red meat (approximately 95 mg/100 g) and chicken (approximately 84 mg/100 g) but the cholesterol content of fish is about 33% lower than that of red meat.

2.4.3 Possible health risks of red meat in the diet

2.4.3.1 General information

Unfortunately, despite its good qualities, the consumption of red meat is regarded by many as bad for one's health and linked to CHD because of its SFA content. It is also associated with cancer, probably because of its fat content (Willet *et al.*, 1990, Giovannucci *et al.*, 1992). Although several studies have shown an association between red meat intake and cancer, it is still not clear whether it is due to the fat content of the meat or to endogenous nitrosamines, carcinogenic tryptophan metabolites, or carcinogens resulting from the cooking of meat (Gerhardsson de Verdier *et al.*, 1991; Zeman, 1991; Giovannucci *et al.*, 1992; Willet *et al.*, 1990). The effect of red meat intake as a risk factor for cancer will not be addressed in this discussion, which focuses on the relationship between red meat intake and CHD.

2.4.3.2 Red meat and coronary heart disease

Snowden *et al.* (1984) have shown a dose-response relationship between meat consumption and CHD risk. Unfortunately in their study there was no differentiation between red meat and poultry consumption. In the Coronary Artery Risk Development in Young Adults (CARDIA) study it was shown that those who ate red meat and poultry less than once a week had lower

TC, LDL-C and TAG levels than those who ate meat more frequently (Slattery *et al.*, 1991). Dietary and lifestyle practices which could have a positive impact on health status were, however, also better in those who ate meat less often.

In a study carried out by the MRC in the South-Western Cape it was shown that red meat was the main dietary source of total fat, SFAs and cholesterol in those who follow a Western type diet (Wolmarans *et al.*, 1989). A high prevalence of hypercholesterolaemia was also found in this population group (Rossouw *et al.*, 1983).

Researchers have shown that a diet high in fat, especially SFAs, is associated with CHD (Keys, 1980) and that SFAs (Mensink, 1993) and dietary cholesterol (Katan *et al.*, 1986) increase plasma TC. Although there is still controversy about the influence of dietary cholesterol on plasma cholesterol, researchers are in agreement that the SFAs myristic and palmitic acids, present in appreciable amounts in red meat, increase plasma TC (See 2.2.3.2 (c)). Although the SFA stearic acid, also present in red meat, does not have a hypercholesterolaemic effect, it is probably thrombogenic (Sinclair, 1982) and could thus play a role in CHD. It should therefore not be ignored in the dietary guidelines for the prevention of CHD.

In a study carried out by the MRC (Wolmarans *et al.*, 1991) it was shown that the levels of the atherogenic lipoproteins, i.e. plasma TC, LDL-C and TAG, were significantly lower on a diet in which fatty fish was the only "meat" in the diet compared to a diet in which red meat was the only "meat" in the diet. Comparison of the nutrient composition of the two diets showed that total fat and SFA intake were significantly lower on the fatty fish diet than on the red meat diet.

2.4.4 Red meat as part of the lipid lowering diet

Several researchers investigated the effect of red meat in the diet on plasma lipids and lipoproteins. These studies are summarised in Table 2.4. In five of the studies the effect of red meat was compared with that of poultry and fish. In four of the five there was no significant difference between the effect of the two diets on TC levels (Flynn *et al.*, 1981; Flynn *et al.*, 1982; O'Brien & Reiser, 1980; Scott *et al.*, 1991). Total cholesterol levels were, however,

significantly lower when fatty fish was consumed instead of red meat which contained fat (Wolmarans *et al.*, 1991). In general, HDL-C showed no change with the treatment diets, but HDL-C was significantly higher on an animal protein diet than on a plant protein diet (Table 2.4). TAG levels were higher on the lean meat diet than on a high fat Australian diet, probably as a result of a higher carbohydrate intake on the lean meat diet (Kestin *et al.*, 1989b). In agreement, O'Dea (1990) also showed an increase in the TAG levels on a very low fat diet.

According to the results summarised in Table 2.4 lean red meat might not have a negative effect on the lipid profile in human subjects.

2.4.5 Conclusion

Red meat has several positive qualities. It is a good source of protein of high biological value, it is an important source of iron, zinc, vitamin B_{12}, niacin, riboflavin and vitamin B_6. Red meat is also a source of many other vitamins and minerals.

Unfortunately red meat is also associated with the development of CHD and cancer, probably because of its fat content. Research has shown that the saturated fatty acids, myristic and palmitic acids, which are present in appreciable amounts in red meat with a high fat content, increase plasma cholesterol levels and could thus contribute to the development of coronary heart disease.

Table 2.4. Studies which investigated the effect of red meat on plasma lipids and lipoproteins

Authors	Study population	Age (years)	Study design	Measurements	Results
O'Brien and Reiser, 1980	29 free-living men TC < 6.2 mmol/L	31-61	Four weeks baseline Four diets six weeks each: *≥170 g/day red meat (beef, pork, mutton) or fish and poultry* (a) red meat plus 3 eggs/day; (b) red meat no eggs; (c) fish and poultry no eggs; (d) fish and poultry plus 3 eggs/day. Group I, 15 subjects consumed diets in the order 1-4. Group II, 14 subjects consumed diets in the order 4-1.	*Diets*: Baseline - seven-day dietary records. Experimental period: daily record of dairy products and egg-containing foods, any kinds of protein and deviations from diet plan. *Blood*: Fasting sample baseline, fifth and sixth week of test period; six weeks after end of study.	No significantly different response to ingestion of red meat versus fish and poultry.
Flynn *et al.*, 1981	74 men 55 women free-living normolipaemic	23-70	Cross-over; no baseline. Two sequential three-month periods. Diets: Self selected plus one egg/day (a) chicken/turkey/fish minimum 5 oz (raw)/day*; *or* (b) beef - minimum 5 oz (raw)/day*. * Provided	*Diet*: Four-day diet records; once in each 3-months experimental period. Nutrient intake calculated. *Blood*: Fasting blood sample 0, 3, 6 months.	*Men*: No difference in TC, TAG and HDL-C. *Women*: No difference in TC and HDL-C, TAG↑ ($p < 0.05$) on poultry and fish.

Table 2.4. (continued)

Authors	Study population	Age (years)	Study design	Measurements	Results
Sacks *et al.*, 1981	14 men 7 women Vegetarians (macrobiotic diet)	20-55	*Control period* (2 weeks) Vegetarian diet - only vegetables. *Experimental period* (4 weeks) - 250 g raw beef added isocalorically to control diet. *Control diet* (2 weeks) Vegetarian diet - only vegetables. Diets consumed under supervision.	*Diet*: Daily 24-hour dietary recall questionnaire. Dietary composition calculated *Blood*: Non-fasting blood sample end of each stage.	**Ingestion of beef**: Plasma TC↑19% ($p<0.01$) above control; HDL-C no change; TC/HDL-C↑. **Beef left out**: Plasma TC and TC/HDL-C back to control values.
Flynn *et al.*, 1982	47 men 29 women free-living normolipaemic	32-62	Cross-over; No baseline; Two sequential three-month periods (a & b). Diets: Self selected diet plus one egg/day (a) beef or (b) poultry + fish (c) 6 weeks *ad libitum* diet after a+b (d) pork (3-months) Minimum 5 oz beef/poultry/fish/pork per day provided.	*Diet*: Four-day dietary records in each experimental period. *Blood*: Fasting blood sample 0, 3, 6 months.	No statistically significant change in serum TC or TAG. HDL-C both ↑&↓.

Table 2.4. (continued)

Authors	Study population	Age (years)	Study design	Measurements	Results
Wiebe *et al.*, 1984	8 men normolipaemic	18-27	Cross-over design. Two sequential 21-day dietary periods - followed in random order: (a) animal protein (APD) - beef protein subsituted for 55% plant protein; (b) plant protein (PPD). Free-living subjects but all meals eaten in Metabolic Laboratory.	*Diets*: Analysed chemically for total lipids. *Blood*: Fasting samples (a) day 1 and 7-day intervals - TC and TAG; (b) days 1, 22 and 43 LDL-C and HDL-C.	Serum TC, LDL-C and TAG did not differ significantly between diets. HDL-C↑($p<0.05$) on APD than PPD.
Watts *et al.*, 1988	15 men free-living hyperlipaemic TC 8.1 mmol/L TAG 3.4 mmol/L	49.9±3.1	Diets: isoenergetic 180 g, meat and meat products per day. *Baseline* - 4 weeks: Diet A (fat meat) 42%E fat; *Experimental* - 2x14 weeks random sequence: Diet B: (very lean meat): 35%E fat Diet C: (very lean meat) 27%E fat - pectin- and gel-forming fibres used as supplements.	*Diet*: Four-day food diary - mid-point of dietary period. *Blood*: weeks 3 and 4	Compared to diet A: Diet B - TC ↓ 8.6%; LDL-C↓11%. Diet C - TC ↓ 18.5%, LDL-C↓23.8%.

Table 2.4. (continued)

Authors	Study population	Age (years)	Study design	Measurements	Results
Kestin *et al.*, 1989b	26 men TC 4.7-9.1 mmol/L	28-64	Respondents randomised in an incomplete block design; two of three diets (six weeks): (a) High fat Australian (AUS); (b) Fat-modified lacto-ovo vegetarian (LOV); (c) 60% of plant protein in LOV replaced with 250 g mainly lean meat (LM) but also chicken. Seven day cycle menu for four energy levels; energy requirements estimated; major sources of protein and fat provided.	*Diet*: Four-day measured food records: (a) pre baseline; (b) during each dietary period. Deviations from diet recorded Food analysis of 2 400 kcal AUS, LOV and LM diets. *Blood*: Fasting sample every two weeks.	**AUS vs LOV**: TC↓10%, LDL-C↓9% ($p<0.001$); HDL-C↓(0.05); TAG↑23% ($p<0.01$) **AUS vs LM**: TC↓5%, LDL-C↓7%, TAG↑24% ($p<0.01$); HDL-C↓($p<0.05$).
O'Dea *et al.*, 1990	5 men 5 women free-living healthy	25±1.9	*Baseline*: One week - normal diet (30-100 g beef/day). *Experimental diet*: (a) Weeks two and three:- Very-low-fat diet (9%E) with 500 g beef.2000kcal.d^{-1}. Pre-packed fat-trimmed beef provided. Carbohydrate supplement 20%E given. (b) Week four:- fat intake ↑ to 20%E, beef dripping replaced one-half of carbohydrate supplement. Week five:- fat intake ↑ to 30%E, beef dripping replaced carbohydrate supplement.	*Diet*: Weighed and recorded all food and liquid for five weeks. Nutrient composition of diet calculated. *Blood*: Fasting sample weekly intervals.	**Very-low-fat diet (week one)**: TC↓, LDL-C↓ ($p<0.001$), TAG↑ ($p<0.03$). **Beef drippings added**: TC↑, LDL-C↑ ($p<0.02$) - (week 5) TAG↓ ($p<0.02$). HDL-C not affected by dietary manipulations.

Table 2.4. (continued)

Authors	Study population	Age (years)	Study design	Measurements	Results
Scott *et al.*, 1991	46 men free-living borderline-high levels of TC	25-55	Three dietary periods: (a) free choice - 3 weeks; (b) isocaloric stabilization - 4 weeks 226 g regular beef; (c) test diets - 4 weeks -randomly asigned: 226 g lean beef or 113 g chicken breast, 113 g fish All food provided during stabilization and test diets. Energy requirements based on three food records in free choice period.	*Diet*: Free choice period - food records, scales provided. Stabilization and test diets. Daily check-lists - reviewed by dietitian. uneaten food returned to clinic. *Blood*: Fasting sample twice before study then weekly.	**Test vs stabilization diet**: *Lean beef*: TC↓0.2%; LDL-C↓-1.1%; HDL-C↓-1.4%; TAG↑10.8%. *Chicken/fish*: TC↓-1.9%; LDL-C↑0.4%; HDL-C↓-6.7%; TAG↑0.2%. **Test diets:** Changes in TC and LDL-C similar in two test diets. HDL-C lower ($p<0.02$) on chicken/fish diet than on lean beef diet.

Table 2.4. (continued)

Authors	Study population	Age (years)	Study design	Measurements	Results
Wolmarans *et al.*, 1991	12 men 16 women TC <8.0 mmol/L	22-45	Cross-over; two phases each with baseline (three weeks), experimental (six weeks), and post-experimental period (six weeks). Three months washout period between phases. *Experimental diets*: Self selected with either (a) red meat (with fat) - beef and mutton (men ≥300 g, raw; women ≥225 g, raw); or (b) fatty fish (men ≥280 g; women ≥216 g, cooked weight). Red meat and fish provided.	*Diets*: Seven-day estimated dietary record in each baseline and experimental period. *Blood*: Fasting sample. Start of baseline, end of baseline and then every three weeks.	Plasma TC, LDL-C, VLDL-C, VLDL-TAG and TAG ↓ on fatty fish than on red meat ($p<0.001$). HDL-C no difference between diets.

CHAPTER 3

MATERIALS AND METHODS[1]

3.1 Introduction

The main aim of this study was to investigate the effect of two prudent diets, which differed only in the type of "meat" (lean red meat versus chicken, without skin, and fish), on the plasma lipids and lipoproteins of free-living subjects with age-related elevated plasma cholesterol levels.

Dietary intervention studies can either be undertaken as inpatient metabolic diet studies or as studies on free-living subjects. Metabolic ward studies are usually well controlled and precise information can be collected (Hegsted *et al.*, 1965) while dietary compliance might be a problem in studies on free-living subjects. It has, however, been shown that non-compliance was not a notable problem in outpatient research studies (Crumb-Johnson *et al.*, 1993). One of the advantages of studying free-living subjects is the fact that they can be studied in their natural environment instead of under artificial conditions. Hypercholesterolaemia is a major problem among many South Africans and studying free-living subjects rather than in a metabolic ward would have been more appropriate.

In this chapter the recruitment of subjects, the study population, the design of the study, the dietary prescription, the measurements, as well as the analysis of the data are described.

3.2 Study population

3.2.1 Recruitment of subjects for the study

Subjects were recruited from the male staff of the MRC, Parow, and from the male and female

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[1] *A multidisciplinary team was responsible for the execution of this study. The names of the team members and their responsibilities are given at the end of this chapter in Annexure 1.*

staff at the head office of SANLAM in Bellville. More than 3 500 letters detailing the aim of the study, criteria for inclusion in the study, as well as how the study would be executed, were distributed among the staff of these organisations (Addendum A).

A fasting blood sample was drawn from 180 people between the ages of 20 and 55 years who were interested to participate in the study. The blood was analysed for TC, HDL-C and TAG. To qualify for the study the age-dependent action limits for TC, as defined by an *ad hoc* committee of the Heart Foundation of Southern Africa, were used (Rossouw *et al.*, 1988). Only those with a moderate or high risk for CHD, in terms of TC, qualified for the study. Seventy of the subjects who met the criteria for TC were willing to participate (Figure 3.1). One of the subjects was slightly below the low risk cut-off point, but was nevertheless included in the study.

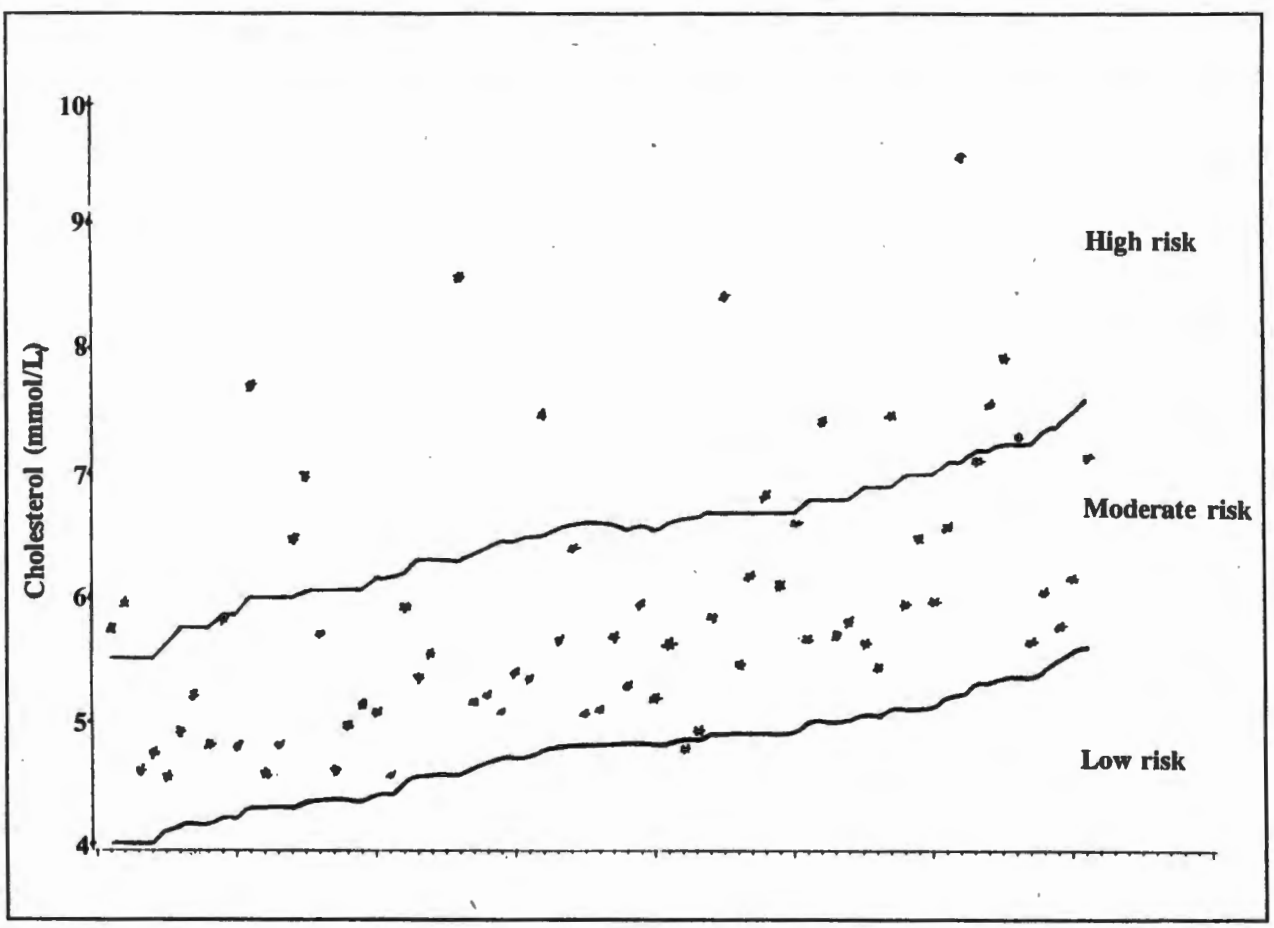

Figure 3.1. **Plasma cholesterol of subjects plotted against age-dependent cholesterol action limits (70 subjects recruited for the study)**

3.2.2 Exclusion criteria for the study

Exclusion criteria for the study were: Diabetes mellitus; a plasma TAG concentration above 4 mmol/L; lipid-lowering, anti-coagulation, and anti-hypertensive treatment; the intake of beta-blockers; a BMI of more than 30 kg/m^2.

3.2.3 Medical evaluation

Before the baseline period of the study, a medical practitioner was responsible for the clinical evaluation of each person who qualified for the study. A blood sample was drawn for the determination of serum urea, creatinine, total protein, albumin, total bilirubin, conjugated bilirubin, alanine transaminase, and gamma-glutamyl transferase as well as for non-fasting blood glucose.

3.2.4 Risk factor questionnaire

A risk factor questionnaire was completed for each subject on the same day that the medical evaluation was performed (Addendum B). Two dietitians were involved in this task.

3.2.5 Characteristics of the study population

Seventy subjects, 36 men, mean age 35.1 years (standard deviation (SD) 7.8), and 34 women, mean age 31.5 years (SD 9.6), participated in the study. General information on the subjects are given in Table 3.1.

3.3 Ethical Approval

The Ethics Committee of the MRC approved the protocol of the study and subjects gave informed written consent to participate in the study (Addendum C).

Table 3.1. General information on subjects at the start of the study

		Men n = 36	Women n = 34
Age (years)	**mean**	35.1	31.5
	SD*	7.8	9.6
Height (meters)	**mean**	1.795	1.669
	SD	0.057	0.068
Body mass (kg)	**mean**	81.0	65.7
	SD	8.9	9.0
Body mass index	**mean**	25.1	23.6
	SD	2.2	3.1
Plasma TC† (mmol/L)	**mean**	5.75	5.65
	SD	0.97	1.25
BP#: Systolic (mm Hg)	**mean**	121.4	114.4
	SD	8.8	7.5
BP: Diastolic (mm Hg)	**mean**	76.6	70.8
	SD	11.2	9.5

* Standard deviation
† Total cholesterol
# Blood pressure

3.4 Design of the study

This clinical trial had a cross-over design and each phase (Phase 1 and Phase 2) had a three-week baseline period, six-week treatment period and six-week post-treatment period with a washout period of approximately two months in-between (Figure 3.2). Subjects were matched for age, BMI and TC and randomly allocated to one of three groups: Study Group 1 (28 subjects) and Study Group 2 (28 subjects) were treatment groups and Study Group 3 (14 subjects) was a reference group.

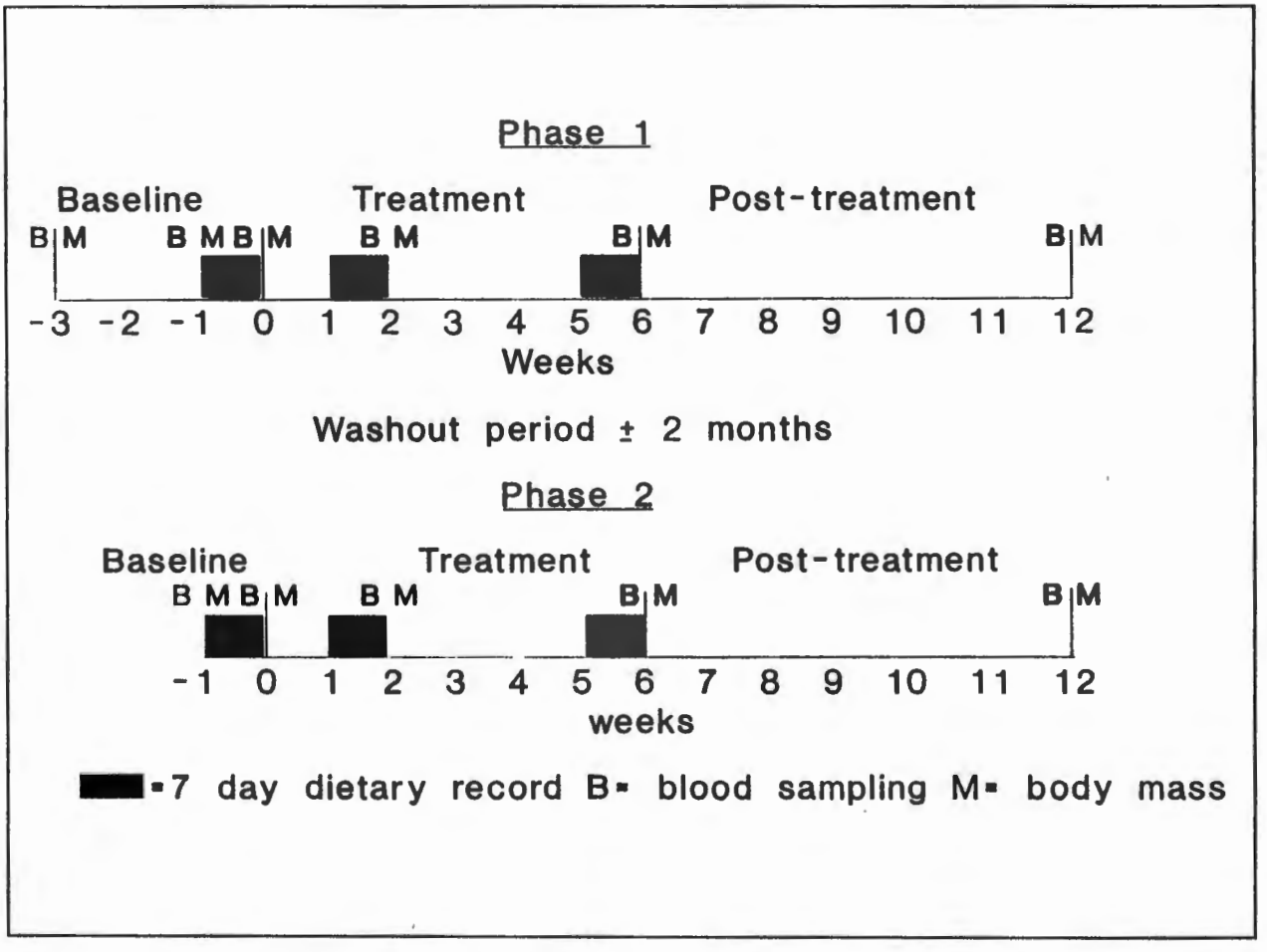

Figure 3.2. **Design of the study**

The measurements taken during the study are summarised in Table 3.2 and will be discussed in more detail later.

Diaries were given to each subject at the start of the baseline periods and at the start of the treatment periods of Phases 1 and 2 (Addendum D). In the diaries appointments for blood sampling, checking of the dietary records, collection of the meat or fish and chicken rations, and the periods for record-keeping were noted. Instructions on medication permitted during the course of the study were also given in the diaries. Subjects were requested to record the use of any medication during the study.

Table 3.2. Measurements taken during Phase 1 and Phase 2 of the study

	Phase 1							Phase 2						
Weeks	**-3**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**12**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**12**
Measurements																			
Height	x																		x
Weight	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Blood pressure	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Seven-day dietary record*			x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x
Dietary compliance questionnaire										x									x

* Dietary records were kept during the last week of the baseline periods and the second and last weeks of the treatment periods.

3.5 Dietary intake during the study

3.5.1 Allocation to diets

During Phase 1, 28 subjects (14 men and 14 women) were allocated to Study Group 1. This group followed the red meat diet (RMD) in Phase 1 and the chicken and fish diet (CFD) in Phase 2. Twenty-eight subjects (15 men and 13 women) were allocated to Study Group 2. This group followed the CFD in Phase 1 and the RMD in Phase 2. The fourteen subjects (7 men and 7 women) of Study Group 3 followed their habitual diet throughout the study (Table 3.3). All the subjects followed their habitual diet during baseline and post-treatment periods (Table 3.3).

Table 3.3. Diets followed by the subjects during the study

Periods	Phase 1	Phase 2
Baseline	Habitual diet (1, 2, 3)*	Habitual diet (1, 2, 3)
Treatment	Red meat (1) Chicken-fish (2) Usual diet (3)	Chicken-fish (2) Red meat (1) Usual diet (3)
Post-treatment	Habitual diet (1, 2, 3)	Habitual diet (1, 2, 3)

* Study Group number in brackets

3.5.2 Calculation of energy needs and food exchanges

The entry weight of subjects and their level of activity were used to calculate their energy requirements (Table 3.4). A series of diets ranging from 7 500 to 14 000 kilojoules (kJ) and differing by approximately 500 kJ, were individually calculated.

Table 3.4. Kilojoule requirements per kilogram body weight and level of activity*

	Level of activity		
	Inactive	**Moderate**	**High**
Kilojoules	125	145	165

* Friedman GJ (1976)

Subjects were given the diet which met their energy needs (Table 3.5). The composition of the diets prescribed during the treatment periods was based on the dietary recommendations for the prevention of CHD (Diet Consensus Panel, 1989). A total fat intake of less than <30%E; a total protein intake of 15%E; a total carbohydrate intake of approximately 55%E; a P/S ratio of 1; and a total dietary cholesterol intake of less than 300 mg per day, were aimed at.

Calculation of the number of food exchanges allowed on the diet was based on the American Exchange List System (American Diabetes Association, American Dietetic Association, 1986). The values used for calculating the different food exchanges are summarised in Table 3.6. Calculation of the number of food exchanges allowed was done according to the method described in the Handbook of Clinical Dietetics (American Dietetic Association, 1981). One and a half milk exchanges and two vegetable exchanges were used throughout the calculations. One and a half milk exchange is 375 mL milk which is in accordance with the recommendation of 400 mL per day for adults (Department of Health Services and Welfare). A summary of the calculated diets is given in Table 3.7.

3.5.3 Dietary prescription

The only difference in the treatment diets was the type of "meat" allowed. Red meat (lean beef and lean mutton) as the only "meat" in the diet was eaten by Study Group 1 in the treatment period of Phase 1 and by Study Group 2 in the treatment period of Phase 2. Chicken without skin and fish (hake, pilchards and tuna) as the only "meat" in the diet were eaten by Study Group 2 in Phase 1 and by Study Group 1 in Phase 2.

Egg intake was restricted to two eggs per week during the treatment periods. Subjects were also requested to avoid hard cheeses during the experimental periods.

The beef, mutton, chicken and fish portions were provided free of charge during the treatment periods but subjects were responsible for providing the remainder of their dietary intake.

In the treatment period every subject in Study Groups 1 and 2 received a personal dietary prescription in the form of a book (Addendum E). This book contained information on the number of food exchanges allowed during the treatment periods, guidelines for the preparation of the food and also the food exchange lists. The NRIND (National Research Institute for Nutritional Diseases) Food Composition Tables (Gouws & Langenhoven, 1986a)[2] and the

[2] *At the time of planning, executing and coding the data of this study, the MRC Food Composition Tables (1991) were not yet available.*

Table 3.5. Energy requirements and energy prescribed during the treatment periods

Men			Women		
Subject No	Energy requirement (kJ)*	Energy prescribed (kJ)	Subject No	Energy requirement (kJ)	Energy prescribed (kJ)
8	8 625	8 379	67	6 625	7 518
187	9 135	9 093	103	7 395	7 518
4	9 570	9 723	49	7 975	8 043
23	9 750	9 723	125	7 975	8 043
185	9 750	9 723	142	7 875	8 043
35	10 150	10 101	55	8 120	8 043
157	10 440	10 815	71	8 265	8 379
20	10 750	10 815	97	8 265	8 379
152	10 585	10 815	100	8 265	8 379
94	10 750	10 815	117	8 410	8 379
186	10 875	10 815	96	8 875	9 093
15	11 310	11 193	69	9 280	9 093
19	11 455	11 193	54	9 250	9 093
24	11 250	11 193	154	9 280	9 093
27	11 310	11 193	58	9 570	9 723
116	11 020	11 193	56	9 715	9 723
5	11 600	11 907	43	9 715	9 723
38	11 600	11 907	75	9 715	9 723
147	11 600	11907	98	9 570	9 734
188	12 325	11 907	156	9 570	9 723
10	12 325	11 907	106	10 295	10 101
11	12 180	11 907	113	10 005	10 101
30	13 050	13 146	93	10 440	10 101
37	13 050	13 146	59	10 440	10 101
48	13 050	13 146	139	10 750	10 815
182	13 195	13 146	148	11 455	11 193
183	13 775	14 007	62	12 035	10 101
128	14 210	14 007			
6	14 790	14 007			

* kilojoules

Table 3.6. Values used for calculating the number of different food exchanges prescribed

Exchanges	Carbohydrate (g)	Protein (g)	Fat (g)	Energy (kJ)*
Milk (low fat)	12	8	5	502
Meat (lean)	-	7	3	230
Bread	15	3	-	335
Fat	-	-	5	188
Fruit	15	-	-	250
Vegetables	5	2	-	105

* kilojoules

Table 3.7. Diets calculated for the treatment periods

	Number of food exchanges					
Energy (kJ)	Milk	Meat	Bread	Fat	Fruit	Vegetables
7 518	1.5	4	10	8	3	2
8 043	1.5	4	11	9	3	2
8 379	1.5	4	12	9	3	2
9 093	1.5	4	13	11	3	2
9 723	1.5	5	14	10	4	2
10 101	1.5	5	14	12	4	2
10 815	1.5	6	16	11	4	2
11 193	1.5	6	16	13	4	2
11 907	1.5	7	18	12	4	2
13 146	1.5	7	20	15	4	2
14 007	1.5	7	22	16	4	2

American Diabetic Exchange List System (American Diabetes Association, American Dietetic Association, 1986) were used to compile the food exchange lists.

3.5.4 Calculation of red meat, chicken and fish rations

During the six weeks of the treatment periods individually pre-packed lean red meat and chicken

(without skin) portions were issued to the subjects weekly. According to the Meat Board the ratio for beef to mutton consumption by South Africans is seven to one (personal communication). Information collected from the subjects (3.2.4 Risk factor questionnaire), however, indicated that mutton was eaten more often per week, and therefore a ratio of beef to mutton of five to two per week was chosen. There are indications that only two servings of fish per week could lower the incidence of mortality from CHD (Kromhout, Bosschieter & Coulander, 1985). A chicken to fish ratio of five to two per week was therefore chosen.

The size of the raw meat and chicken portions required to supply the number of exchanges are given in Tables 3.8 and 3.9.

Table 3.8. Size (in grams) of the raw beef and mutton portions required to supply the prescribed number of exchanges

	Number of exchanges			
	4	**5**	**6**	**7**
Lean beef mince (g)	160	210	250	290
Low fat sausage (g)	145	180	215	255
Rump steak (g)	110	145	180	215
Chuck cubes (g)	110	145	180	215
Topside strips (g)	145	180	215	255
Leg of lamb chops (g)	145	180	215	250
Leg of lamb (g)	145	180	215	250

Calculations for the beef and mutton portions were based on a moisture loss of between 20 and 30%. In the case of chicken, calculations were based on a moisture and bone loss of 50% for thighs and drumsticks and a moisture loss of 20% for breasts.

Frozen hake, tuna canned in water, and pilchards canned in tomato sauce were given with the chicken. Every subject received enough frozen hake at the start of the treatment period to supply one portion (required number of exchanges) of fish per week for six weeks. The tuna and pilchards were issued alternatively to supply the second portion of fish per week.

Table 3.9. Size (in grams) of the raw chicken portions required to supply the prescribed number of exchanges

	Number of exchanges			
	4	**5**	**6**	**7**
Breasts (g)	135 g x 2	180 g x 2	225 g x 2	270 g x 2
Thighs (g)	180 g x 1	225 g x 1	270 g x 1	315 g x 1
Drumsticks (g)	180 g x 2	225 g x 2	270 g x 2	315 g x 2

3.5.5 Guidelines for the preparation of meat and chicken

Guidelines for the preparation of the meat, chicken and fish were issued with the rations in weeks one and two of the treatment periods (Addendum F).

3.5.6 Evaluation of dietary compliance

A questionnaire on dietary compliance (Addendum G) was completed at the end of the cross-over study.

3.6 Measurements

3.6.1 Dietary data

3.6.1.1 Collection of dietary information

Dietary information was collected by means of seven-day dietary records. Dietary records were kept once during the baseline period (last week of baseline period) and twice during the treatment period (second and sixth week of treatment period) of Phases 1 and 2. Subjects were instructed on record- keeping during a special lecture. Each subject received a "Weigh Less" scale for weighing food portions and was shown how to use the scale. Subjects were also

instructed on how to keep record in household measures when they were unable to weigh the food, e.g. when they went out for a meal. A booklet with written instructions on record-keeping was also given to each subject (Addendum H). Subjects were requested to give tradenames where applicable and to describe salads, casseroles, etc. in as much detail as possible or to provide the recipe. Two dietitians were responsible for checking the dietary records after two days and again after completion of the seven-day dietary records. Plastic food models, household spoons, metric spoons and measuring cups as well as a ruler were used to estimate portion sizes where information was incomplete. The dietary records kept during the second week of the treatment period were used for collection of dietary data and also to check for compliance with the dietary prescription.

3.6.1.2 Coding of the dietary data

The NRIND Food Composition Tables (FCT) were used to code the food for the type of food eaten (Gouws & Langenhoven, 1986a). When the food eaten was not weighed on the scale provided, the NRIND Food Quantities Manual (FQM) was used to convert the amount of food reported in household and metric measures or in terms of dimensions, to grams of food eaten (Langenhoven *et al.*, 1986). The encoded information was computerised for analysis of the data.

3.6.2 Anthropometric data

3.6.2.1 Determination of body mass

Body mass in light clothing without shoes was measured on an ordinary bathroom scale to the nearest 0.5 kg. The same scale was used each time and adjusted to zero before each subject was weighed.

3.6.2.2 Determination of height

Height without shoes was measured to the nearest 0.001 meters with a special tape measure device fitted to a wall.

3.6.3 Measurement of blood pressure

Blood pressure and pulse rates were taken after the subjects had been seated for at least five

minutes. Only three previously standardised observers were used for blood pressure measurement.

Blood pressure was measured by means of a 12.5 x 23 cm inflatable cuff connected to a vertical mercury manometer (Model 300 Baumanometer, WA Baum, NY). The systolic pressure was taken at the beginning of phase 1, i.e. the pressure at which the first Korotkoff sound is heard in the brachial artery when the cuff is deflated at a rate of two to four mm Hg per heart beat, after the cuff was initially inflated to a pressure of 30 mm Hg higher than was necessary to totally block the palpable arterial flow in the arm. The diastolic pressure was taken at the beginning of phase V, i.e. that pressure at which the last Korotkoff sound is heard by means of a standard clinical stethoscope when the cuff was deflated at a constant rate as stated above. Three blood pressure measurements were taken and the lowest pressure was recorded.

3.6.4 Blood sampling and analyses

3.6.4.1 Blood sampling

Five minutes after taking the blood pressure and pulse rates, stasis-free, fasting (12 hours) blood samples were taken from the antecubital vein of a subject's arm into evacuated glass tubes. For kidney and liver function analyses, a 10 mL serum separating tube was used. For lipid indices, 8 mL evacuated ethylenediamine-tetra-acetic acid (EDTA)$[K]_3$ (1 mg/mL blood) tubes were used.

3.6.4.2 Analysis of blood samples

(a) ***Plasma total cholesterol***

The high performance CHOD-PAP enzymatic colorimetric kit of Boehringer Mannheim, Cat No 237574, was used to determine plasma TC. For control of accuracy Precinorm^R L was used as the external control. Interlab pooled serum was used as the internal control. Boehringer Mannheim calibrator for automated systems 759350 was used to calibrate the RA1000 auto-analyzer.

(b) ***High density lipoprotein cholesterol***

In order to separate the high density lipoprotein fraction, the apo B-containing lipoproteins, VLDL, IDL and LDL, were precipitated by adding manganese chloride/heparin ($MnCl_2$/heparin)

to the plasma (Gidez *et al.*, 1982). The high performance CHOD-PAP enzymatic colorimetric kit of Boehringer Mannheim, Cat No 237574, was then used to determine HDL-C.

(c) ***High density lipoprotein$_3$-cholesterol***

The HDL fraction was obtained by using $MnCl_2$/heparin to precipitate the apo B-containing lipoproteins. The HDL_2 was precipitated with dextran sulphate (MW=15 000) to separate HDL_3 (Gidez *et al.* 1982). The HDL_3-C was determined by the high performance CHOD-PAP enzymatic colorimetric kit of Boehringer Mannheim (Cat No 237574).

(d) ***High density lipoprotein$_2$-cholesterol***

HDL_2-C was determined by using the formula:

HDL_2-C = HDL-C - HDL_3-C (Gidez *et al.*, 1982)

(e) ***Very low density lipoprotein plus intermediate density lipoprotein and low density lipoprotein fractions***

The VLDL and IDL fractions were isolated together as one fraction by preparative ultracentrifugation at a density of d = 1.019 in a titanium Beckman 40.3 rotor at 40 000 rpm and 10°C for 20 hours. LDL_1 (d = 1.019-1.030 g/mL) and LDL_2 (d = 1.030-1.063 g/mL) were isolated under the same conditions. The enzymatic colorimetric method (Boehringer Mannheim Cat No 237574) was used for the determination of VLDL + IDL-cholesterol as well as for LDL_1-C and LDL_2-C. VLDL-TAG was determined by an enzymatic colorimetric method (Boehringer Mannheim Cat No 701904).

(f) ***Triacylglycerol***

TAG was determined by the high performance enzymatic colorimetric test of Boehringer Mannheim Cat No 701904. For control of accuracy Precinorm^R L was used. Boehringer Mannheim calibrator for automated systems 759350 was used to calibrate the RA1000 auto-analyzer.

(g) ***Apolipoprotein B in low density lipoprotein 1 and 2 cholesterol***

A nephelometer was used for the turbidimetric determination of apo B in LDL_1 and LDL_2, using Boehringer Mannheim anti-human apo B antiserum (Cat No 726494).

(h) ***Plasma cholesteryl ester and triacylglycerol fatty acid composition***

Plasma (300 μL) was extracted according to the method of Folch, Lees & Stanley (1957) with chloroform-methanol (2:1 vol/vol). Butylated hydroxytoluene was used as anti-oxidant. The TAG and CE were separated by thin-layer chromatography (TLC) and the spots containing esterified cholesterol (EC) and TAG were scraped off and transmethylated by heating with 2.5 mL methanol/18 mol/L sulphuric acid (95:5; v/v) for 2 hours at 70°C as described by Benadé *et al.* (1988).

A Varian 3700 gas chromatograph (GC) equipped with a flame ionization detector and a Varian CDS 402 data system were used to analyse fatty acid methyl esters extracted with hexane. Separation was done with the GC using 30 m fused silica megabore DB-225 columns of 0.53 mm internal diameter (J & W Scientific, Cat No 125-2232). The following conditions were used: gas flow rates for hydrogen (carrier gas) were 5-8 mL/min; for medical air, 250 mL/min; and for hydrogen 25mL/min. The injector temperature was 240°C and the detector temperature 250°C. As standard, a fatty acid mixture prepared from individual fatty acids (Sigma, St. Louis, MO, USA) was used. Fatty acid methyl esters of CE and TAG were identified by comparison with the retention times of those of the standard mixture of fatty acids C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:3, C20:4, C20:5, C22:5 and C22:6 (Smuts *et al.*, 1992).

A list of the common names of the fatty acids and the number of carbon atoms in each chain is given in Annexure 2.

3.7 Statistical analysis of the project

Analysis of the dietary, biochemical and anthropometric data was done by the Centre for Epidemiological Research in Southern Africa (CERSA) of the MRC using the Statistical Analysis System (SAS, Volumes 1 and 2, 1989).

3.7.1 Processing and statistical analysis of dietary data

3.7.1.1 Energy and nutrients

The computerised NRIND FCT (Gouws & Langenhoven, 1986a) was used for the analysis of the seven day dietary records. Dietary intakes were analysed for energy, macronutrients, dietary cholesterol and dietary fibre intake and the P/S ratio of the diet was also calculated.

To give an indication of the cholesterol-elevating effect of the diet, the Keys dietary score (Shekelle *et al.*, 1981) was calculated for the baseline and treatment (sixth week) periods:

$$\text{Keys dietary score} = 1.26(2S-P)+1.5(1000C/E)^{½}$$

where S = SFAs and P = PUFAs as a percentage of total energy; C = dietary cholesterol in mg/day and E = daily energy intake in kilocalories.

The Keys equation was calculated to predict the expected change in plasma TC between the baseline periods and the treatment periods (week six of treatment period) which could be expected as a result of dietary intervention: Keys equation: Δchol = expected change in total cholesterol (mg/100 mL); ΔS = difference in the percentage of total energy from SFAs between the baseline and treatment periods; ΔP = difference in the percentage of total energy from PUFAs between the baseline and treatment period; ΔZ = difference in dietary cholesterol, mg/1000 kcal, between the baseline and treatment period (Keys, Anderson & Grande, 1965b).

3.7.1.2 Food groups

Foods consumed during the seven-day dietary record periods were classified into different food groups, i.e. milk, meat, bread, fat, fruit, and vegetables, based on Langenhoven *et al.* (1989). The number of red meat, chicken, fish, egg, milk, red meat plus eggs, white meat (fish and chicken) plus eggs, bread, fat, fruit and vegetable exchanges was calculated (Langenhoven *et al.*, 1989). Calculation of the different exchanges were based on the following:

milk group:	gram protein from milk foods/8 = 1 milk exchange;
meat group:	gram protein/7 = 1 meat exchange;
	gram protein/6 = 1 exchange (one egg);
bread:	gram carbohydrate/15 = 1 bread exchange;

fat: gram total fat/5 = 1 fat exchange;

fruit: gram (carbohydrate - sugar)/15 = 1 fruit;

vegetables: gram (carbohydrate - sugar)/5 = 1 vegetable exchange.

3.7.1.3 Dietary compliance

In order to determine dietary compliance, differences were calculated:

(i) between dietary energy prescribed and actual energy intake;

(ii) between the food exchanges prescribed and the food exchanges actually consumed.

The correlation between energy prescribed and actual energy intake (seven-day dietary records) was also calculated.

3.7.2 Processing of biochemical data

Blood samples were collected twice during the baseline and treatment periods (Figure 3.2). The results for plasma lipids and lipoproteins and fatty acid composition of the CE and plasma TAG, however, only represent the blood samples collected at the end of the baseline periods and at the end (week six) of the treatment periods.

3.7.3 Processing of anthropometric data

BMI was calculated as weight (kg)/height $(m)^2$.

3.7.4 Statistical analysis of dietary and biochemical data

The statistical analysis used in this project was very specific to the design of the study. This was a 2x2 cross-over trial plus a reference group which received no treatment in both periods. The 2x2 trial consisted of two treatments and two periods. The treatment effects are estimated within subjects rather than between subjects and since the variability of measurements taken on different subjects is usually far greater than the variability of repeated measurements taken on the same subject this design is statistically more powerful and optimal. This is the main advantage of the cross-over trial.

In a 2x2 cross-over trial carry-over effects and direct treatment effect-by-period interaction cannot be distinguished from each other. When carry-over effects were found in some variables the clinical implications of the effect were investigated to see if they were in fact influencing the results in a clinical way. This was done since our direct treatment comparisons were done under the assumption of *no* second-period baseline differences or direct treatment by period interaction.

The baseline information used in the analysis to increase the power of the statistical test was the measurement taken at the start of each active treatment period. The final measurements of the active treatment periods were used for assessing treatment effects. Four repeated measurements of each individual were therefore used:
Baseline (1) - third week of the baseline period;
Treatment (1) - sixth week of the treatment period;
Baseline (2) - the week (only one) of the baseline period;
Treatment (2) - sixth week of the treatment period.

The three contrasts considered for analysis of the comparison of the treatments were:
(a) ½(1, 0, -1, 0) for checking the comparability of the three groups at the time of the second baseline measurement;

(b) ½(1, -1, 1, -1) to examine direct treatment-by-period interactions for the two active treatments;

(c) ¼ (0, -1, 0, 1) for estimating and testing the direct treatment effect of the active treatments subject to the condition that (a) and (b) do not show any significant differences.

The above-mentioned methods are suggested by Jones & Kenward (1989) in their book *Design and Analysis of Cross-Over Trials*.

For testing and estimating the difference in treatment effects of the active treatments versus reference, the period effect was assumed to be zero and the relevant treatments from both periods were combined to give a two-sample comparison of reference versus one of the treatments. For the reference group (Study Group 3) the 'treatment effect' was averaged over

both periods.

The standardised range test of Tukey was used for the baseline comparisons and the t-test was used for all the other two sample comparisons.

The change from each baseline was also computed for each individual and the mean changes estimated plus the necessary standard deviations.

ANNEXURE 1

Execution of the study - tasks and people responsible

TASK	PERSON/LABORATORY MAINLY RESPONSIBLE*	CO-WORKERS
Organisation		
Study leader (responsible for overall organisation and execution of study)	Ms P Wolmarans[1]	
Study protocol and ethical approval	Ms P Wolmarans[1]	Dr AJS Benadé[1] Dr CJ Lombard[2] Dr JA Kriek[1]
Recruitment of volunteers	Ms P Wolmarans[1]	Dr AJS Benadé[1] Sr L Marx[3]
Medical evaluation of subjects	Dr JA Kriek[1]	
Questionnaire development		
Risk Factor Questionnaire	Ms P Wolmarans[1]	
Questionnaire to measure dietary compliance	Ms P Wolmarans[1]	
Collection of questionnaire information	Ms P Wolmarans[1] Mrs S van der Merwe[4]	
Dietary prescription		
Calculation of energy requirements; Calculation of meat, chicken and fish portions.	Mrs S van der Merwe[4] Ms P Wolmarans[1] (supervisor)	
Compilation of Exchange List and diet plan	Ms P Wolmarans[1]	
Documentation for seven-day dietary records	Ms P Wolmarans[1]	
Compilation of diaries for the study	Ms P Wolmarans[1]	

Annexure 1. (continued)

TASK	PERSON/LABORATORY MAINLY RESPONSIBLE	CO-WORKERS
Collection of dietary data		
Training of subjects in record keeping.	Ms P Wolmarans[1]	
Checking of dietary records	Ms P Wolmarans[1] Mrs S van der Merwe[4]	
Compilation of hints for the preparation of meat and chicken	Mrs S Van der Merwe[4]	
Anthropometric measurements	Ms P Wolmarans[1] (height) Mrs S vd Merwe[4] and Sr L Marx[3] (weight)	
Measurement of blood pressure	Dr JA Kriek[1] Dr MA Dhansay[1] Mr C de W Marais[1]	
Blood sampling	Dr JA Kriek[1], Dr MA Dhansay[1], Mr C de W Marais[1]	
Analysis of blood samples		
Plasma lipids and lipoproteins	Mr M Marais[1]	
Fatty acids	Mrs S vd Merwe[4], Mr G Engelbrecht[1]	Mr HY Tichelaar[1] (supervisor)
Analysis of blood sample for serum urea, creatinine, total protein, albumin, total bilirubin, conjugated bilirubin, alanine transaminase, gamma-glutamyl transferase and non-fasting blood glucose.	Department of Chemical Pathology, Tygerberg Hospital	

Annexure 1. (continued)

TASK	PERSON/LABORATORY MAINLY RESPONSIBLE	CO-WORKERS
Statistical analysis of the project	Mrs R Laubscher[2] Dr CJ Lombard[2]	Ms P Wolmarans[1] (specific requests for analysis of the data)

* The "Methods" used in the study are described in the text of Chapter 3

[1] National Research Programme for Nutritional Intervention, Medical Research Council, P O Box 19070, TYGERBERG 7505.

[2] Centre for Epidemiological Research in Southern Africa, Medical Research Council P O Box 19070, TYGERBERG 7505. (Dr CJ Lombard was responsible for the statistcal design of the study)

[3] SANLAM, P O Box 1, SANLAMHOF, 7532

[4] At the time of the study Mrs S vd Merwe was a MRC bursary recipient and registered for the M-Nutrition degree at the University of Stellenbosch

ANNEXURE 2

Common names of fatty acids and the number of carbon atoms

Common name of fatty acid	Number of carbon atoms
Butyric acid	C4:0
Caproic acid	C6:0
Caprylic acid	C8:0
Capric acid	C10:0
Lauric acid	C12:0
Myristic acid	C14:0
Myristoleic acid	C14:1
Palmitic acid	C16:0
Palmitoleic acid	C16:1
Stearic acid	C18:0
Oleic acid	C18:1
Linoleic acid	C18:2
Linolenic acid	C18:3
Stearidonic acid	C18:4
Arachidic acid	C20:0
Gadoleic acid	C20:1
Eicosatrienoic acid	C20:3
Arachidonic acid	C20:4
Timnodonic acid (Eicosapentaenoic acid)	C20:5
Behenic acid	C22:0
Erucic acid (cis-Δ^{13}-docosenoic acid) **and** Cetoleic (Δ^{11}-docosenoic acid)	C22:1
Clupanodonic acid (Docosapentaenoic acid)	C22:5
Docosahexaenoic acid	C22:6
Lignoceric acid	C24:0

Reference: Diem K & Lentner C, eds (1975): Scientific Tables. Documenta Geigy. 7th ed. Basle: Ciba-Geigy Limited, 366-370.

CHAPTER 4

RESULTS AND DISCUSSION OF DIETARY COMPLIANCE AND DIETARY INTAKES

4.1 Introduction

Dietary intervention is the first step in the treatment of hypercholesterolaemia and the main aim with the diet is to lower fat intake, especially saturated fat intake (Adult Treatment Panel II, 1994). In order to lower fat intake the restriction of red meat often forms part of the dietary recommendations to lower plasma cholesterol since red meat usually has a high fat content and the fat consists mainly of saturated fatty acids. South Africans who follow a Western type diet have a preference for red meat (Wolmarans *et al.*, 1989). The question arises whether red meat should be excluded or could form part of a lipid-lowering diet for people with elevated plasma cholesterol levels. This situation is especially relevant in the South African situation where there is a high prevalence of hypercholesterolaemia (Rossouw *et al.*, 1983) among those who follow a Western type diet (Wolmarans *et al.*, 1988). The aim of this study, therefore, was to investigate the effect of two prudent diets, which differed only in the type of "meat" (lean red meat versus chicken, without skin, and fish), on plasma lipids and lipoproteins of free-living subjects with age-related elevated plasma cholesterol levels.

In contrast with other studies on free-living subjects in which virtually all the food for the treatment periods was provided (Flaim *et al.*, 1981; Mensink & Katan, 1987), in this study only the lean red meat, chicken without skin, and fish portions were provided free of charge. Subjects were responsible for providing the remainder of the food themselves during the test periods.

It is easier to compare the effects of individual foods or nutrients on health markers in metabolic ward studies than in studies on free-living subjects. Test diets can be prepared and fed under strict control to subjects who are studied in metabolic wards. It is, however, much more difficult to control for confounding dietary variables in free-living subjects when the effect on

health issues of different food items or specific nutrients are compared. Dietary compliance is therefore one of the major issues that has to be addressed when a study on free-living subjects is undertaken since non-compliance could jeopardise the results of a study. Test diets for this study were planned individually and based on energy needs and prudent dietary guidelines (Diet Consensus Panel, 1989) in order to keep the diets as comparable as possible and to ensure compliance with the dietary prescription. (The compilation of and execution of the test diets are discussed in more detail in Chapter 3).

Dietary compliance can either be determined subjectively by responses to particular questions in connection with compliance, or by collecting dietary intake information, or a bio-marker could be used to assess intakes over a specific period.

Several methods are available for collecting information on dietary intake (Bingham *et al.*, 1988) e.g. the 24-hour recall, the dietary history, the food frequency questionnaire and the dietary record method. The 24-hour recall, the dietary history and the food frequency questionnaire are recall methods which depend on the memory of the subject being studied. These methods are used to collect different types of dietary information. To collect information on food intake on a specific day the 24-hour recall method is used; the dietary history is used to determine habitual food intake over a period of time and the food frequency assesses how often specific foods are eaten (Bingham *et al.*, 1988). The 24-hour recall is used for the collection of food consumption data of a group of individuals (Bingham *et al.*, 1988) and the information is only considered valid if the method is used to describe the mean intake of a group of at least 50 subjects or more (Block, 1982). Precise information on the weight of foods eaten and drunk are required for research purposes and a recall method depends too much on the memory of the study participant. The weighed record is usually the method of choice for research purposes since it is regarded as the most accurate method of dietary assessment (Bingham *et al.*, 1988). The weighed record, nevertheless, also has its limitations. The respondent's burden could result in a change of food intake (Bingham *et al.*, 1988). This limitation is especially important when habitual food intake is studied.

The method of choice for the collection of dietary data during this study was therefore a combination of the weighed plus estimated seven-day dietary record method. If food consumed could not be weighed, e.g. when subjects ate away from home, portion sizes were described in

household or metric measures or information on the dimensions of food was given (see Chapter 3). One of the main reasons for choosing the seven-day dietary record method was to collect more precise information on the weight of the foods consumed. The seven-day dietary record was also used to check compliance with the dietary prescription. Every time the records were checked the importance of dietary compliance to the outcome of the study was stressed. The keeping of the seven-day dietary records could cause respondent burden. This could then result in a change of food intake and was especially addressed when the dietary records were checked.

Since this study was performed on free-living subjects, special attention was given to two aspects, namely: (a) the compilation of the test diets in order to control for confounding dietary variables; and (b) the collection of dietary intake data for evaluating dietary compliance and reporting energy and nutrient intake. Results on dietary compliance and the intake of energy and nutrients during the study will be reported and discussed in this chapter.

4.2 Results

4.2.1 Number of subjects who completed the study

Sixty-three of the 70 subjects originally recruited for the study completed Phase 1 of the cross-over study. One of the subjects withdrew for health reasons and six of the subjects could not see their way clear to follow the therapeutic diet. Fifty-four subjects completed Phase 1 and Phase 2 of the cross-over study. Reasons for not completing Phase 2 of the study were: three participants (two women, one man) moved from the Cape Peninsula, one man could not continue with the study for health reasons, one woman could not complete the study for personal reasons, and one woman went on a holiday and did not want to follow the treatment diet during this period. Three women were not motivated to follow the treatment diet again in Phase 2. Since one of the women who completed Phase 2 lost too much weight during the study her data was not included in the analyses. One of the subjects in the reference group had incomplete data for Phase 2. Complete biochemical and anthropometric data were therefore available for 52 subjects. Since the seven-day dietary records of two of the subjects were not reliable their dietary data were not included in the dietary analyses. Results on dietary intake are therefore

given for only 50 subjects, 37 of whom formed part of the treatment groups, and 13 control subjects.

4.2.2 Compliance with dietary prescription

Dietary compliance was determined by means of dietary intake, a questionnaire as well as a biomarker. The latter will be discussed in Chapter 6.

Table 4.1 gives the difference in prescribed and measured energy intakes.

Table 4.1. Mean difference between dietary energy prescribed and actual energy intake (mean of seven-day dietary record) during the cross-over study*

	Phase 1			Phase 2		
	B_1†	T_1#	T_2§	B_1†	T_1#	T_2§
Number of subjects	**19**	**19**	**19**	**18**	**18**	**18**
Study Groups						
Group 1						
mean (kJ)¶	557.3	2856.4**	2808.5**	2048.5††	2747.5##	2868.3**
SD§§	2130.7	2026.4	1879.2	3093.3	2602.8	2276.7
Group 2						
mean (kJ)	1120.7	3040.3**	3643.1**	1652.0##	3505.2**	3287.4**
SD	2660.5	1634.1	2116.9	2220.7	1854.0	2221.1

* Difference = {energy prescribed (kJ) - actual energy (kJ) intake}
† Last week of baseline period
# Second week of treatment period
§ Sixth week of treatment period
¶ Kilojoule
Significantly different from baseline: ** $p < 0.001$; †† $p < 0.05$; ## $p < 0.01$
§§ Standard deviation

Results of the seven-day dietary records show that except for the baseline period of Phase 1, mean energy intakes were significantly lower than the dietary energy prescribed.

Significant differences between the energy intakes in the baseline and treatment periods were observed and the mean differences are reported in Table 4.2. In Phase 2 no difference in energy intake between the baseline and the sixth week of the treatment period was observed in Study Group 2.

Table 4.2. Mean difference between baseline energy intake and energy intake during the treatment periods

	Phase 1		Phase 2	
	T_1*	T_2†	T_1*	T_2†
Study Group 1 (n = 19)				
mean (kJ)#	0.29	0.43	0.34	0.36
SD§	0.54	0.66	0.54	0.62
p-value	0.0457	0.0141	0.0180	0.0253
Study Group 2 (n = 18)				
mean	0.27	0.50	0.29	0.15
SD	0.40	0.45	0.54	0.62
p-value	0.0174	0.0005	0.0422	0.3300

* Second week of treatment period
† Sixth week of treatment period
# Kilojoule
§ Standard deviation

The mean number of meat, chicken, fish and egg exchanges consumed from the meat food group per day, during the treatment periods, is summarised in Table 4.3. During T_1 of Phase 1 the mean portion of chicken plus fish (Study Group 2) consumed was slightly smaller (5.71) than the mean portion of lean red meat (5.89) consumed by Study Group 1. One meat exchange is equal to 30 g which means that the chicken plus fish portion was 5 g smaller than the lean red meat portion. In T_2 of Phase 1 the chicken and fish portion was 26 g smaller, in T_1 of Phase 2 it was 24 g smaller, and in T_2 of Phase 2 it was 14 g bigger. The daily mean intake of eggs varied between 0.14 and 0.28 which is equal to approximately 0.98 and 1.96 eggs per week. Egg intakes were restricted to two eggs per week. The majority of subjects had a mean intake of between one and two eggs per week during the treatment periods. Tables 4.4 and 4.5 show

Table 4.3. The mean (and standard deviation) number of red meat, chicken and fish exchanges and the number of eggs consumed daily during the treatment periods*

		Phase 1				Phase 2			
		Treatment 1†		Treatment 2#		Treatment 1		Treatment 2	
		Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
No of subjects		**19**	**18**	**19**	**18**	**19**	**18**	**19**	**18**
Red meat	**mean**	5.89	0.07	5.61	0.0	0.04	5.87	0.04	5.23
	SD§	1.61	0.30	1.40	0.0	0.16	2.81	0.16	1.85
Chicken	**mean**	0.00	3.71	0.03	3.33	3.35	0.02	3.85	0.05
	SD	0.00	1.28	0.10	1.22	0.72	0.07	1.14	0.14
Fish	**mean**	0.11	2.00	0.03	1.4	1.73	0.01	1.85	0.02
	SD	0.32	0.81	0.0	1.00	0.72	0.03	2.16	0.07
Eggs	**mean**	0.22	0.21	0.27	0.19	0.14	0.16	0.21	0.28
	SD	0.19	0.23	0.16	0.14	0.14	0.16	0.17	0.30

* Calculated from the seven-day dietary records kept during the treatment periods. § Standard deviation
† Second week of treatment period
# Sixth week of treatment period

Table 4.4. Difference between food exchanges prescribed and food exchanges actually consumed by subjects of Study Group 1 (n = 19)*

Periods	Milk	Red meat + eggs	White meat + eggs	Bread	Fat	Fruit	Vegetables
Phase 1							
T_1†							
mean	0.57	-0.74		9.07	5.65	1.63	0.22
SD#	0.47	0.80		3.03	2.82	1.23	0.56
p-value	0.0001	0.0004		0.0001	0.0001	0.0001	0.0971
T_2§							
mean	0.47	-0.51		9.13	5.71	1.77	0.47
SD	0.47	1.12		2.27	2.31	1.06	0.75
p-value	0.0007	0.0919		0.0001	0.0001	0.0001	0.02
Phase 2							
T_1							
mean	0.58		0.15	9.69	6.27	1.05	0.35
SD	0.56		0.64	2.48	2.27	1.72	1.03
p-value	0.001		0.4615	0.0001	0.0001	0.0258	0.0506
T_2							
mean	0.5		-0.27	9.43	6.01	1.32	0.34
SD	0.43		2.42	2.71	2.47	1.69	0.81
p-value	0.0002		0.8674	0.0001	0.0001	0.0033	0.0679

* Exchanges prescribed - exchanges consumed
† T_1 = week two of treatment period
# Standard deviation
§ T_2 = week six of treatment period

Table 4.5. Difference between food exchanges prescribed and food exchanges actually consumed by subjects of Study Group 2 (n = 18)*

Periods	Milk	Red meat + eggs	White meat + eggs	Bread	Fat	Fruit	Vegetables
Phase 1							
T_1†							
mean	0.28		-0.59	8.79	5.01	1.84	0.40
SD#	0.64		1.11	2.93	2.81	1.10	0.68
p-value	0.0837		0.335	0.0001	0.001	0.0001	0.0244
T_2§							
mean	0.40		0.42	9.13	5.35	1.96	0.24
SD	0.51		1.46	3.04	2.82	0.91	1.03
p-value	0.0046		0.2788	0.0001	0.0001	0.0001	0.2080
Phase 2							
T_1							
mean	0.35	-0.70		8.99	5.21	1.52	0.54
SD	0.65	2.41		3.06	3.28	1.83	0.69
p-value	0.0537	0.1674		0.0001	0.0001	0.0066	0.0040
T_2							
mean	0.39	-0.17		9.15	5.37	1.67	0.45
SD	0.66	1.52		3.10	2.99	1.43	1.21
p-value	0.0264	0.5798		0.0001	0.0001	0.0004	0.1272

* Exchanges prescribed - exchanges consumed
# Standard deviation
† T_1 = week two of treatment period
§ T_2 = week six of treatment period

the difference between the food exchanges prescribed and the food exchanges actually consumed by Study Groups 1 and 2. The number of lean red meat plus egg exchanges was significantly higher than prescribed, but only in Study Group 1.

Results show that the number of food exchanges consumed was generally less than the number prescribed. This was especially true for the number of fat exchanges prescribed. The number of meat exchanges consumed was, however, slightly higher than the number prescribed.

Compliance with the dietary prescription for the treatment periods was also evaluated by means of a questionnaire. The response rate on the questionnaire was 94.6% (35 of 37 subjects on treatment). Table 4.6 shows that 34 (97.1%) of the subjects were of the opinion that they complied with 70% or more of the dietary prescription. Only three (8.6%) of the subjects indicated that they had violated the prescription for alcohol consumption more than three times per week.

Table 4.6. Self scoring by subjects of their dietary compliance (n = 35)

	Number of Subjects	
	Red meat diet	**Chicken and fish diet**
Score out of ten		
4	1	
6		1
7	10	4
8	3	4
9	13	17
10	8	9

Figure 4.1, however, shows that compliance with the dietary prescription for the meat exchanges was better in Phase 1 than in Phase 2. Compliance with the dietary prescriptions for the milk, fat, vegetables and fruit exchanges was also better in Phase 1 than in Phase 2 (Figure 4.1). Seventeen (48.6%) of the 35 subjects who completed the questionnaire on dietary compliance indicated that they did not eat either red meat or chicken and fish when it was not prescribed.

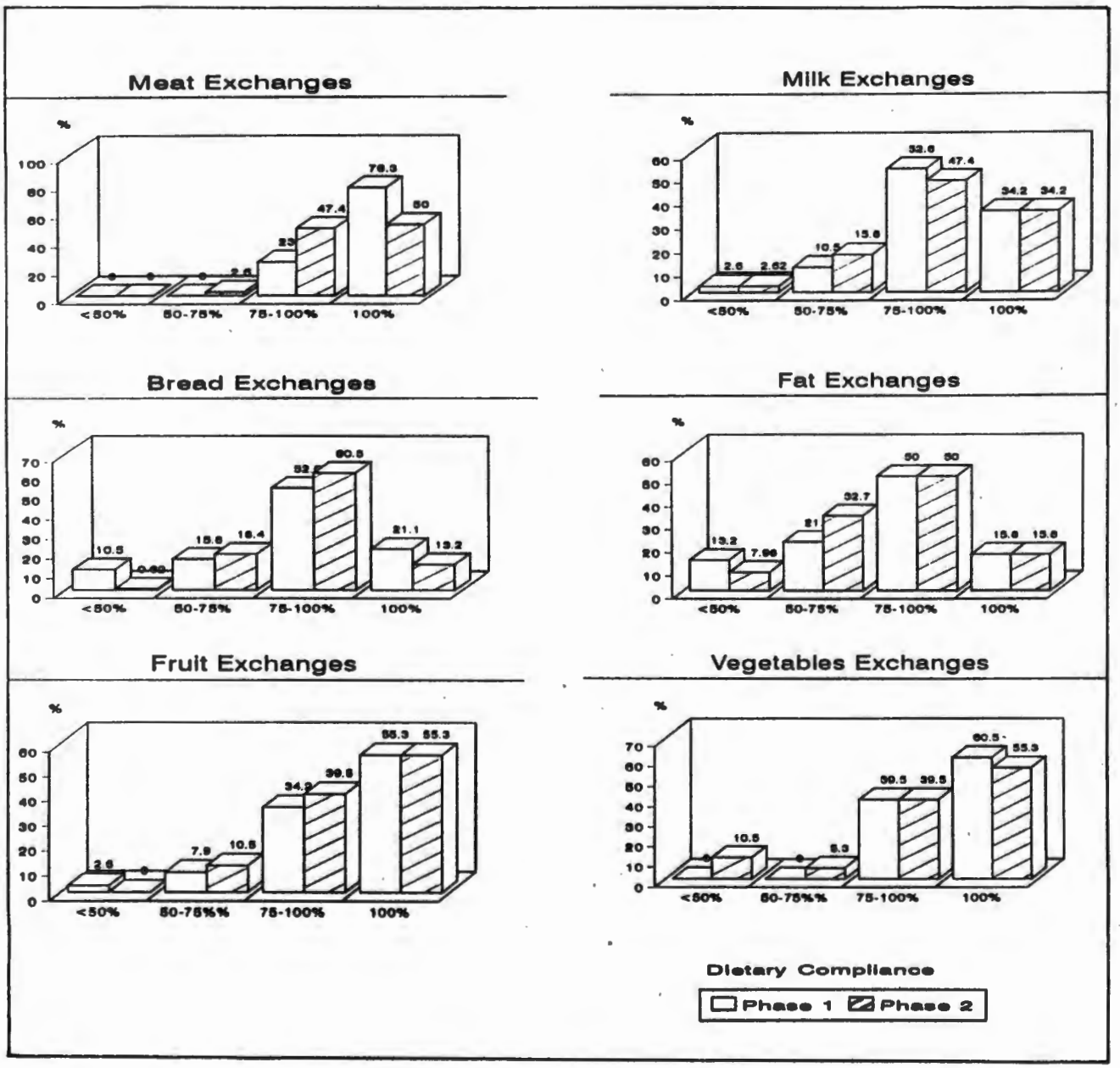

Figure 4.1. Percentage of subjects who complied with the prescription for the different food exchanges

Table 4.7 shows the number of times chicken and fish was eaten on the RMD or red meat was eaten on the CFD. Eight (22.9%) of the subjects violated the prescription of not to eat red meat on the CFD while 13 (37.1%) ate chicken and/or fish when it was not prescribed. Six of the 13 subjects ate chicken and fish, five ate chicken and two ate fish on the RMD.

Table 4.7. Number of subjects who ate either red meat or chicken or fish when it was not prescribed

	Number of times during treatment period				
	0	1	2	3	6
	Number of subjects				
Red meat	27	4	3	1	0
Chicken	24	3	7	1	0
Fish	27	4	1	2	1

In Table 4.8 the number of times per week that subjects would have liked to consume red meat, chicken and fish is given.

Table 4.8. Number of times per week that subjects would like to consume red meat, chicken or fish

	Red meat	Chicken	Fish
Times per week	Percentage of subjects		
0	0	0	2.6
1	18.4	15.8	47.4
2	28.9	44.7	36.8
3	26.3	26.3	10.5
4	21.1	7.9	2.6
5	5.3	5.3	0

A small percentage (5.3%) of the subjects indicated that they would have liked to eat red meat five times per week. Approximately one-fifth of the subjects indicated that they would have liked to eat red meat four times per week while this figure for chicken and fish was 7.9% and 2.6% respectively. Only 55.3% of the subjects indicated that the portion of fish given was just right and 26.3% felt that the portion of fish was too small.

A large percentage (81.6%) of the subjects indicated that they would be willing to accept the new dietary pattern if they were allowed to eat lean red meat as well as chicken (without skin) and fish.

4.2.3 Mean dietary intakes

Mean values and standard deviations for energy, macronutrients, dietary cholesterol and dietary fibre intake, as well as the dietary P/S ratio and the Keys dietary score for Study Groups 1 and 2 (treatment groups) and Study Group 3 (reference group), are given in Tables 4.9, 4.10 and 4.11 for the baseline periods and T_2 of Phases 1 and 2. In Study Group 1 (Table 4.9) total fat and dietary cholesterol intake were significantly higher in the baseline period for RMD than in the baseline period for CFD while in Study Group 2 no significant baseline differences were observed for the CFD and the RMD (Table 4.10). In Study Groups 1 and 2 total fat (%E), saturated fat (%E) and monounsaturated fat (%E) intakes as well as the Keys dietary score were significantly higher on the RMD than on the CFD (Tables 4.9 and 4.10). In Study Group 2 energy and dietary cholesterol intakes were also significantly higher on the RMD than on the CFD (Table 4.10). The P/S ratio in Study Groups 1 and 2, carbohydrate intake in Study Group 1, and polyunsaturated fat intake in Study Group 2 were significantly lower on the RMD than on the CFD. In Study Group 3 the only significant difference was in energy intake between the two baseline periods (Table 4.11).

In Figure 4.2 the percentage of energy provided by lean red meat, chicken (without skin) and fish is shown for Study Groups 1, 2 and 3 for the different 7-day dietary record periods. In Study Groups 1 and 2 the percentage of energy provided by chicken (without skin) plus fish in the test diet (T_1 and T_2) was lower than the percentage of energy provided by lean red meat in the test diet (T_2).

Table 4.9. Mean (and standard deviation) energy and macronutrient intake, P/S ratio and Keys dietary score of subjects in the baseline and treatment periods (Study Group 1, n = 19)

		Diets				Difference	
		Phase 1		Phase 2			
		Red meat		Chicken-Fish		Base-line*	T_2†
Dietary variables		**B#**	**T_2§**	**B#**	**T_2§**	**p-values**	
Energy (kJ)¶	**mean**	9 739.8	7 506.6	8 667.0	6 904.9	0.066	0.196
	SD**	2 216.5	2 162.9	2 505.4	2 348.0		
Protein (%E)††	**mean**	14.8	17.3	15.0	17.4	0.738	0.984
	SD	2.5	2.7	2.9	3.6		
Carbohydrate (%E)	**mean**	45.2	52.8	48.4	56.3	0.060	0.049
	SD	5.6	4.8	7.6	5.2		
Fibre (g)	**mean**	18.4	22.0	18.7	20.2	0.829	0.098
	SD	6.4	8.1	8.8	8.3		
Total fat (%E)	**mean**	37.6	28.7	35.4	25.8	0.029	0.014
	SD	4.7	3.9	4.1	2.7		
Saturated fat (%E)	**mean**	13.0	8.4	11.9	6.3	0.104	<0.001
	SD	2.7	1.0	1.9	0.8		
Monounsaturated	**mean**	13.6	9.9	12.8	7.7	0.374	<0.001
fat (%E)	**SD**	2.3	1.5	2.1	1.1		
Polyunsaturated	**mean**	7.9	7.8	7.9	9.1	0.829	0.060
fat (%E)	**SD**	2.2	2.6	1.2	1.7		
Dietary cholesterol (mg)	**mean**	314.6	219.9	274.6	186.6	0.018	0.192
	SD	101.0	55.7	101.2	76.0		
P/S ratio##	**mean**	0.63	0.93	0.68	1.46	0.418	<0.001
	SD	0.22	0.30	0.16	0.27		
Keys dietary score§§	**mean**	40.2	28.0	37.2	22.1	0.156	<0.001
	SD	7.6	4.8	6.1	5.6		
Alcohol (%E)	**mean**	4.29	3.62	3.25	2.75	0.159	0.107
	SD	4.31	3.60	3.25	3.10		

* Difference between baseline periods Phases 1 and 2
** Standard deviation
† Difference between treatment periods Phases 1 and 2
†† Percentage of total energy
# Baseline period
Polyunsaturated to saturated fatty acid ratio
§ T_2 week six of treatment period
§§ Keys dietary score (Shekelle *et al.*, 1981)
¶ Kilojoules

Table 4.10. Mean (and standard deviation) energy and macronutrient intake, P/S ratio and Keys dietary score of subjects in the baseline and treatment periods (Study Group 2, n = 18)

		Diets				Difference	
		Phase 1		Phase 2			
		Chicken-Fish		Red meat		Base-line*	T_2†
Dietary variables		**B#**	**T_2§**	**B#**	**T_2§**	**p-values**	
Energy (kJ)¶	**mean**	9 170.9	6 949.8	8 293.8	7 483.7	0.167	0.018
	SD**	3 404.5	2 233.1	3 621.1	2 861.8		
Protein (%E)††	**mean**	14.9	18.0	15.9	17.0	0.067	0.347
	SD	2.5	2.8	2.7	2.2		
Carbohydrate (%E)	**mean**	45.3	53.0	47.7	50.0	0.523	0.196
	SD	7.8	4.9	10.5	8.7		
Fibre (g)	**mean**	17.0	20.8	20.6	19.3	0.678	0.186
	SD	6.4	8.5	13.8	8.8		
Total fat (%E)	**mean**	38.2	28.0	35.4	31.0	0.054	0.003
	SD	5.0	4.1	6.7	6.0		
Saturated fat (%E)	**mean**	12.6	6.7	11.8	9.3	0.074	0.001
	SD	2.4	0.9	3.4	2.1		
Monounsaturated	**mean**	13.6	8.0	12.5	10.6	0.417	<0.001
fat (%E)	**SD**	2.4	1.0	2.8	2.3		
Polyunsaturated	**mean**	8.8	10.2	8.0	8.4	0.265	0.006
(fat %E)	**SD**	2.8	3.0	3.0	2.5		
Dietary cholesterol (mg)	**mean**	285.6	188.0	281.8	218.0	0.710	0.005
	SD	143.3	70.8	168.2	117.0		
P/S ratio##	**mean**	0.73	1.54	0.73	0.93	0.832	<0.001
	SD	0.26	0.45	0.32	0.27		
Keys dietary score§§	**mean**	37.6	19.1	37.3	30.2	0.865	<0.001
	SD	9.3	6.7	11.9	8.7		
Alcohol (%E)	**mean**	3.11	2.83	2.86	3.06	0.854	0.639
	SD	3.11	3.07	3.61	3.35		

* Difference between baseline periods Phases 1 and 2
† Difference between treatment periods Phases 1 and 2
\# Baseline period
§ T_2 week six of treatment period
¶ Kilojoules
** Standard deviation
†† Percentage of total energy
Polyunsaturated to saturated fatty acid ratio
§§ Keys dietary score (Shekelle *et al.*, 1981)

Table 4.11. Mean (and standard deviation) energy and macronutrient intake, P/S ratio and Keys dietary score of subjects in the baseline and treatment periods (Study Group 3, n = 13)

		Diets				Difference	
		Phase 1		Phase 2			
		Habitual		Habitual		Base-line*	T2†
Dietary variables		B#	T_2§	B#	T_2§	p-values	
Energy (kJ)¶	mean	10 033.4	8 690.0	8 709.4	8 889.2	0.040	0.826
	SD**	3 796.0	2 720.3	2 641.9	2 763.8		
Protein (%E)††	mean	15.5	15.9	15.9	17.0	0.636	0.581
	SD	2.5	2.5	2.7	2.2		
Carbohydrate (%E)	mean	44.1	43.8	45.8	43.3	0.455	0.588
	SD	10.8	8.3	11.6	8.3		
Fibre (g)	mean	21.6	18.7	18.0	18.6	0.168	0.791
	SD	8.8	6.0	8.6	7.9		
Total fat (%E)	mean	34.5	34.0	31.8	34.1	0.234	0.893
	SD	6.0	5.5	6.5	6.1		
Saturated fat (%E)	mean	11.4	11.6	10.7	11.7	0.340	0.685
	SD	1.9	2.3	3.1	3.0		
Monounsaturated	mean	12.7	12.3	11.1	12.0	0.244	0.636
fat (%E)	SD	3.5	3.2	3.3	3.1		
Polyunsaturated	mean	7.5	7.1	6.8	7.4	0.787	0.685
fat (%E)	SD	1.7	1.1	2.0	1.9		
Dietary cholesterol (mg)	mean	315.5	305.5	282.5	319.1	0.251	0.600
	SD	162.8	148.9	147.0	170.2		
P/S ratio##	mean	0.67	0.64	0.70	0.70	1.00	1.000
	SD	0.19	0.21	0.34	0.38		
Keys dietary score§§	mean	36.3	38.0	35.6	37.7	0.946	0.946
	SD	6.1	7.5	9.1	11.6		
Alcohol (%E)	mean	8.00	7.90	8.90	8.49	0.850	0.898
	SD	7.16	7.91	8.32	8.23		

* Difference between baseline periods Phases 1 and 2
† Difference between treatment periods Phases 1 and 2
\# Baseline period
§ T_2 week six of treatment period
¶ Kilojoules
** Standard deviation
†† Percentage of total energy
Polyunsaturated to saturated fatty acid ratio
§§ Keys dietary score (Shekelle *et al.*, 1981)

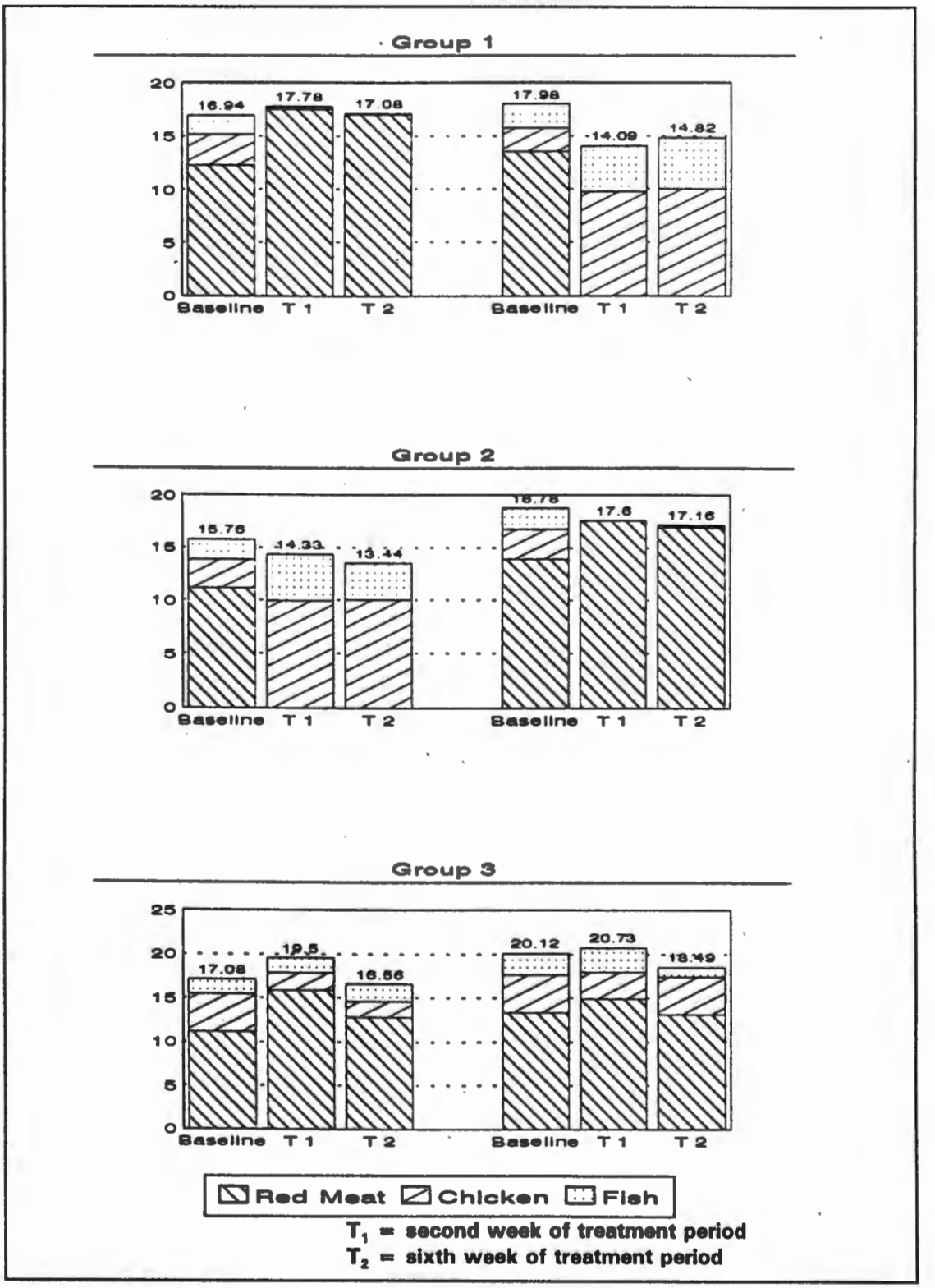

Figure 4.2. Percentage of energy from red meat, chicken and fish

The mean intakes of fatty acids are reported in Tables 4.12, 4.13 and 4.14 for Study Groups 1, 2 and 3.

Table 4.12. Mean (and standard deviation) dietary intake of fatty acids in the baseline and treatment periods (Study Group 1, n = 19)

	Diets							
	Phase 1				Phase 2			
	Red Meat				Chicken and Fish			
	B_1*		T_2†		B_1*		T_2†	
Fatty acids (gram)	Mean	SD#	Mean	SD	Mean	SD	Mean	SD
C4:0	0.49	0.32	0.17	0.15	0.35	0.15	0.162	0.09
C6:0	0.28	0.19	0.10	0.09	0.19	0.09	0.10	0.05
C8:0	0.23	0.22	0.06	0.05	0.14	0.07	0.06	0.03
C10:0	0.43	0.32	0.14	0.11	0.28	0.14	0.13	0.07
C12:0	1.05	1.80	0.21	0.13	0.52	0.33	0.19	0.08
C14:0	2.64	1.37	1.16	0.59	2.03	0.65	0.76	0.41
C14:1	0.28	0.15	0.15	0.06	0.17	0.06	0.03	0.05
C16:0	15.83	4.33	8.46	3.27	13.32	3.90	6.54	2.69
C16:1	1.67	0.63	0.85	0.30	1.36	0.61	0.57	0.25
C18:0	7.95	2.44	4.42	1.67	6.87	2.17	2.98	1.36
C18:1	29.38	8.69	15.73	6.25	24.48	7.03	12.35	5.30
C18:2n-6	13.69	4.93	12.07	5.69	14.47	6.78	13.55	6.03
C18:3n-3	0.70	0.22	0.54	0.25	0.67	0.22	0.29	0.14
C18:4	0.01	0.02	<0.01	0.0	0.01	0.01	0.01	0.01
C20:0	0.15	0.06	0.09	0.05	0.12	0.06	0.10	0.06
C20:1	0.13	0.11	0.05	0.03	0.11	0.09	0.11	0.05
C20:3	0.03	0.05	<0.01	0.0	0.03	0.05	0.02	0.03
C20:4n-6	0.14	0.06	0.10	0.03	0.12	0.08	0.12	0.04
C20:5n-3	0.05	0.08	<0.01	<0.01	0.06	0.10	0.11	0.10
C22:0	0.19	0.09	0.14	0.08	0.16	0.09	0.15	0.10
C22:1	0.65	1.79	0.10	0.40	0.03	0.06	0.14	0.40
C22:5n-3	0.02	0.03	<0.01	0.0	0.02	0.03	0.04	0.03
C22:6n-3	0.09	0.13	<0.01	<0.01	0.13	0.19	0.26	0.26
C24:0	0.06	0.04	0.05	0.03	0.05	0.03	0.05	0.04

* Last week of baseline period
† Week six of treatment period
\# Standard deviation

Table 4.13. Mean (and standard deviation) dietary intake of fatty acids in the baseline and treatment periods (Study Group 2, n = 18)

	Diets							
	Phase 1				Phase 2			
	Chicken and fish				Red Meat			
	B_1*		T_2†		B_1*		T_2†	
Fatty acids (gram)	Mean	SD#	Mean	SD	Mean	SD	Mean	SD
C4:0	0.48	0.27	0.15	0.08	0.36	0.23	0.20	0.17
C6:0	0.27	0.16	0.09	0.05	0.20	0.13	0.12	0.10
C8:0	0.19	0.12	0.06	0.03	0.14	0.09	0.08	0.07
C10:0	0.39	0.23	0.13	0.07	0.30	0.17	0.18	0.14
C12:0	0.70	0.53	0.20	0.13	0.59	0.58	0.31	0.33
C14:0	2.37	1.09	0.67	0.35	2.13	1.64	1.32	0.75
C14:1	0.26	0.15	0.02	0.02	0.17	0.10	0.16	0.08
C16:0	14.75	5.71	5.74	2.22	13.03	7.97	9.39	4.23
C16:1	1.47	0.60	0.46	0.19	1.31	0.75	0.90	0.45
C18:0	7.15	2.68	2.58	1.04	6.79	5.12	5.07	2.26
C18:1	27.97	13.37	11.15	4.77	23.27	14.04	17.54	8.10
C18:2n-6	15.69	8.53	13.43	5.74	12.79	5.61	13.71	6.75
C18:3n-3	0.64	0.25	0.42	0.26	0.70	0.60	0.48	0.23
C18:4	<0.01	0.01	0.01	0.02	0.01	0.01	<0.01	<0.01
C20:0	0.17	0.16	0.09	0.04	0.10	0.05	0.09	0.04
C20:1	0.14	0.11	0.11	0.07	0.09	0.05	0.05	0.02
C20:3	0.02	0.03	0.01	0.02	0.03	0.06	<0.01	0.01
C20:4n-6	0.13	0.08	0.10	0.04	0.12	0.08	0.10	0.05
C20:5n-3	0.04	0.04	0.10	0.09	0.05	0.07	<0.01	0.01
C22:0	0.24	0.27	0.14	0.07	0.13	0.06	0.13	0.07
C22:1	0.31	0.57	0.12	0.39	0.17	0.58	0.10	0.28
C22:5n-3	0.01	0.01	0.04	0.03	0.02	0.02	<0.01	<0.01
C22:6n-3	0.07	0.08	0.22	0.17	0.09	0.12	0.01	0.03
C24:0	0.08	0.10	0.05	0.03	0.04	0.02	0.05	0.03

* Last week of baseline period
† Week six of treatment period
\# Standard deviation

Table 4.14. Mean (and standard deviation) dietary intake of fatty acids in the baseline and treatment periods (Study Group 3, n = 13)

	Diets							
	Phase 1				Phase 2			
	Habitual				Habitual			
	B_1*		T_2†		B_2*		T_2†	
Fatty acids	Mean	SD#	Mean	SD	Mean	SD	Mean	SD
C4:0	0.47	0.35	0.39	0.20	0.47	0.39	0.43	0.38
C6:0	0.26	0.20	0.21	0.11	0.26	0.23	0.24	0.20
C8:0	0.22	0.19	0.14	0.06	0.19	0.15	0.16	0.13
C10:0	0.42	0.29	0.31	0.13	0.38	0.32	0.33	0.27
C12:0	0.90	0.88	0.42	0.19	0.69	0.67	0.61	0.63
C14:0	2.42	1.47	1.95	0.88	2.26	1.54	2.43	1.87?
C14:1	0.27	0.21	0.22	0.17	0.25	0.22	0.26	0.19
C16:0	15.69	8.63	12.45	6.67	12.73	7.01	13.89	8.25
C16:1	1.72	1.18	1.29	0.69	1.37	0.74	1.60	0.79
C18:0	7.64	4.01	6.26	3.16	6.21	3.68	6.83	4.23
C18:1	29.49	17.02	23.02	13.42	22.57	12.78	24.03	15.03
C18:2n-6	15.29	7.95	11.13	6.42	11.56	6.54	11.47	7.05
C18:3n-3	0.89	0.59	0.68	0.28	0.64	0.33	0.74	0.34
C18:4	<0.01	<0.01	<0.01	0.01	<0.01	0.01	<0.01	<0.01
C20:0	0.15	0.09	0.12	0.10	0.12	0.12	0.11	0.10
C20:1	0.20	0.16	0.10	0.07	0.13	0.10	0.12	0.08
C20:3	0.03	0.04	0.03	0.03	0.04	0.05	0.04	0.03
C20:4n-6	0.18	0.15	0.12	0.08	0.12	0.05	0.12	0.06
C20:5n-3	0.04	0.04	0.04	0.05	0.05	0.06	0.03	0.03
C22:0	0.21	0.13	0.16	0.14	0.18	0.24	0.16	0.16
C22:1	0.03	0.04	0.03	0.03	0.03	0.04	0.02	0.02
C22:5n-3	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.01
C22:6n-3	0.10	0.09	0.08	0.08	0.10	0.11	0.07	0.06
C24:0	0.06	0.05	0.05	0.05	0.06	0.10	0.05	0.06

* Last week of baseline period
† Week six of treatment period
\# Standard deviation

Tables 4.12 and 4.13 show that the mean intakes of the SFAs, myristic, palmitic and stearic acids were higher on the RMD than on the CFD in Study Group 1 and in Study Group 2. Oleic acid intake was also higher on the RMD than on the CFD. In Study Groups 1 and 2 arachidonic acid intake was approximately the same on the CFD and the RMD. In Study Group 3 (Table 4.14) the figures for arachidonic acid intake were in agreement with those of Study Groups 1 and 2. The mean EPA in the baseline periods varied between 0.04 and 0.06 g per day (Tables 4.12, 4.13 and 4.14). The mean intake of EPA on the RMD was less than 0.01 g per day.

4.2.4 Baseline versus treatment periods - energy and macronutrients

Significant differences between the baseline periods and the treatment periods were found for the RMD and the CFD. Energy intake, total fat (%E), saturated fat (%E), monounsaturated fat (%E) and dietary cholesterol intake were significantly lower on the RMD than on the baseline diet (Table 4.15). Protein (%E) and carbohydrate (%E) intake as well as the P/S ratio of the diet were significantly higher on the RMD than on the baseline diet while fibre intake and polyunsaturated fat (%E) intake did not differ significantly (Table 4.15). Results observed for the CFD were in agreement with that of the RMD. Fibre intake (in gram) and polyunsaturated fat (%E) intake, however, were also significantly higher on the CFD than on the baseline diet (Table 4.15).

4.2.5 Baseline versus treatment periods - fatty acids

Table 4.16 shows that the intake of the majority of fatty acids was significantly lower on the treatment diets than on the baseline diets. The intake of linoleic acid, however, did not differ between the baseline and treatment diets. EPA intake was significantly higher on the CFD than on the baseline diet. Arachidonic acid intake was significantly lower on the RMD than on the baseline diet while no significant difference was observed between the CFD and the baseline diet.

Table 4.15. Difference in energy and macronutrient intake, P/S ratio and Keys dietary score between the baseline period and treatment period (treatment period - baseline period).

Dietary variables	Red meat diet			Chicken and fish diet		
	Mean* n = 37	SD†	p-value	Mean n = 37	SD	p-value
Energy (kJ)#	-1 540.9	1 567.6	0.0001	-1 985.4	1 788.0	0.0001
Protein (%E)§	1.8	2.9	0.0002	2.78	3.2	0.0001
Carbohydrate (%E)	5.0	8.2	0.0003	7.8	5.9	0.0001
Fibre (g)	1.3	7.3	0.1394	2.6	6.1	0.0121
Total fat (%E)	-6.7	6.3	0.0001	-9.9	4.2	0.0001
Saturated fat (%E)	-3.6	3.4	0.0001	-5.7	2.0	0.0001
Monounsaturated fat (%E)	-2.8	2.6	0.0001	-5.3	2.1	0.0001
Polyunsaturated fat (%E)	0.1	2.7	0.6411	1.3	2.5	0.0051
P/S ratio¶	0.25	0.34	0.0001	0.80	0.39	0.0001
Dietary cholesterol (mg)	-79.7	99.1	0.0001	-92.7	73.8	0.0001
Keys dietary score**	-9.7	10.7	0.0001	-16.8	8.8	0.0001
Alcohol (%E)	-0.25	2.5	0.8551	-0.4	2.3	0.4194

* Negative result indicates that baseline value is higher than treatment value
† Standard deviation
# Kilojoules
§ Percentage of total energy intake
¶ Polyunsaturated to saturated fatty acid ratio
** Keys dietary score (Shekelle *et al.*, 1981)

Table 4.16. Difference in fatty acid intake between the baseline period and treatment period (treatment period - baseline period)

	Red meat diet			Chicken and fish diet		
Fatty acids (gram)	**Mean* n=37**	**SD†**	**p-value**	**Mean n=37**	**SD**	**p-value**
C4:0	-0.245	0.307	0.0001	-0.257	0.209	0.0001
C6:0	-0.131	0.174	0.0001	-0.138	0.119	0.0001
C8:0	-0.115	0.179	0.0001	-0.105	0.096	0.0001
C10:0	-0.205	0.288	0.0001	-0.206	0.176	0.0001
C12:0	-0.569	1.395	0.0001	-0.411	0.400	0.0001
C14:0	-1.159	1.427	0.0001	-1.478	0.825	0.0001
C14:1	-0.068	0.148	0.0127	-0.188	0.131	0.0001
C16:0	-5.560	5.334	0.0001	-7.862	4.024	0.0001
C16:1	-0.618	0.635	0.0001	-0.898	0.587	0.0001
C18:0	-2.653	3.300	0.0001	-4.220	1.995	0.0001
C18:1	-9.792	9.724	0.0001	-14.412	8.901	0.0001
C18:2n-6	-0.387	5.399	0.6517	-1.572	7.019	0.2408
C18:3n-3	-0.183	0.360	0.0027	-0.297	0.272	0.0001
C18:4	-0.006	0.013	0.0059	0.008	0.016	0.0044
C20:0	-0.035	0.061	0.0011	-0.053	0.110	0.0009
C20:1	-0.067	0.083	0.0001	-0.015	0.098	0.7376
C20:3	-0.029	0.054	0.0001	-0.013	0.050	0.0750
C20:4n-6	-0.026	0.060	0.0319	-0.011	0.082	0.8916
C20:5n-3	-0.044	0.071	0.0001	0.057	0.119	0.0006
C22:0	-0.025	0.079	0.1149	-0.058	0.194	0.0732
C22:1	-0.315	1.402	0.0053	-0.036	0.518	0.7522
C22:5n-3	-0.018	0.023	0.0001	0.024	0.031	0.0001
C22:6n-3	-0.087	0.126	0.0001	0.142	0.255	0.0001
C24:0	-0.003	0.030	0.9085	-0.016	0.074	0.3720

* Negative result indicates that baseline value is higher than treatment value.
† Standard deviation

4.2.6 Cross-over analysis

The p-values for the differences between the baseline comparisons (carry-over effect) and the direct-by-period interaction effect are given in Table 4.17 for energy and macronutrient intakes of the two treatment groups. No significant differences for the baseline comparisons were observed for energy and macronutrient intake which indicated no first-order carry-over effect at the time of the second baseline period. There were also no difference in treatment carry-over at the time of the second treatment measurement which is called the direct-by-period interaction effect.

Table 4.17. P-values for the differences between the baseline comparisons (carry-over effect) and the direct-by-period interaction effect for Study Groups 1 and 2: energy and macronutrient intake, P/S ratio and Keys dietary score (n = 37)

Dietary variables	Baseline comparisons (Carry-over effect)	Direct-by-period interaction effect
	p-value	p-value
Energy (kJ)*	0.7759	0.2192
Protein (%E)†	0.2596	0.7036
Carbohydrate (%E)	0.7379	0.1463
Fibre (%E)	0.2984	0.4034
Total fat (%E)	0.7505	0.1739
Saturated fat (%E)	0.6580	0.2915
Monounsaturated fat (%E)	0.8076	0.2709
Polyunsaturated fat (%E)	0.3576	0.6162
Dietary cholesterol (mg)	0.1947	0.6639
P/S ratio§	0.6259	0.7500
Keys dietary score¶	0.3285	0.7603
Alcohol (%E)	0.4771	0.2713

* Kilojoules
† Percentage of total energy intake
§ Polyunsaturated to saturated fatty acid ratio
¶ Keys dietary score (Shekelle *et al.*, 1981)

In Table 4.18 baseline comparisons and the direct-by-period interaction effect for fatty acid intakes are given. No significant differences in fatty acid intakes for the baseline comparisons

and the direct-by-period interaction effect were observed.

Table 4.18. P-values for the differences between the baseline comparisons (carry-over effect) and the direct-by-period interaction effect for Study Groups 1 and 2: dietary fatty acids (n = 37)

Fatty acids (gram)	Baseline comparisons (carry-over effects)	Direct-by-period interaction effect
	p-value	p-value
C4:0	0.7758	0.9237
C6:0	0.7619	0.9193
C8:0	0.4517	0.5261
C10:0	0.5052	0.5871
C12:0	0.3706	0.3978
C14:0	0.3780	0.6987
C14:1	0.7892	0.9602
C16:0	0.5891	0.5492
C16:1	0.4983	0.5828
C18:0	0.4792	0.4363
C18:1	0.9454	0.4994
C18:2n-6	0.0720	0.7033
C18:3n-3	0.5038	0.5548
C18:4	0.3051	0.3081
C20:0	0.1858	0.9807
C20:1	0.4494	0.8032
C20:3	0.5758	0.6235
C20:4n-6	0.8007	0.7174
C20:5n-3	0.8581	0.6563
C22:0	0.1536	0.5132
C22:1	0.3110	0.6911
C22:5n-3	0.7180	0.5793
C22:6n-3	0.8477	0.8377
C24:0	0.1745	0.6202

4.2.7 Direct treatment effect

The direct treatment effect refers to the effect of the RMD and the CFD, within the cross-over design, on dietary intake. The intake of energy, total fat expressed as a percentage of total energy intake (%E), total saturated fatty acid (%E), total monounsaturated fatty acids (%E), and dietary cholesterol, as well as the Keys dietary score, were significantly higher on the RMD than on the CFD (Table 4.19). Carbohydrate intake (%E), polyunsaturated fatty acid intake (%E), and the P/S ratio of the diet were significantly higher on the CFD than on the RMD. The difference in protein, fibre and alcohol intake between the two diets was not significant (Table 4.19). The contrast as given in the statistical section, 3.7, was used for estimating the direct treatment effect. A negative difference between the mean contrasts of the two groups indicates that the value for the RMD was higher than for the CFD.

In Table 4.20 the direct treatment effect between the RMD and the CFD is given for the fatty acids. Significant differences were observed between the RMD and the CFD. A negative difference between the mean contrasts of the two groups indicates that C14:0, C14:1, C16:0, C16:1, C18:0, C18:1 and C18:3 for the RMD was higher than for the CFD. Results show that the value for the long chain fatty acids, C20:5, C22:5 and C22:6, were significantly higher on the CFD than on the RMD.

4.2.8 Treatment diet versus reference diet

In Tables 4.21 and 4.22 the differences between the dietary changes for macronutrients in the RMD and the reference diet, and between the CFD and the reference diet, are given. Estimated differences in fatty acids between the changes in the RMD and the reference diet, and between the CFD and the reference diet, are also given in Tables 4.23 and 4.24. Significant differences between the RMD and the reference diet as well as between the CFD and the reference diet were observed. Differences between the CFD and the reference diet seemed to be more significant than between the RMD and the reference diet. Changes for linoleic acid and EPA intake did not differ between the RMD and the reference diet and the CFD and the reference diet. A negative result indicates that the variable decreased while a positive result indicates that the variable increased over time on the treatment diet.

Table 4.19. Direct treatment effect between red meat diet and chicken-fish diet: energy and macronutrient intake, P/S ratio and Keys dietary score (n = 37)*

Dietary variable	Lower confidence limit	Difference between means†	Upper confidence limit	p-value
Energy (kJ)#	-504.0	-284.0	-64.0	0.0131
Protein (%E)§	-0.3	0.3	0.8	0.3286
Carbohydrate (%E)	0.7	1.6	2.5	0.0008
Fibre (g)	-0.9	-0.1	0.7	0.8087
Total fat (%E)	-2.0	-1.5	-1.0	0.0001
Saturated fat (%E)	-1.4	-1.2	-1.0	0.0001
Monounsaturated fat (%E)	-1.5	-1.2	-0.9	0.0001
Polyunsaturated fat (%E)	0.4	0.8	1.1	0.0001
P/S ratio¶	0.22	0.28	0.35	0.0001
Dietary cholesterol (mg)	-25.0	-15.8	-6.7	0.0012
Keys dietary score**	-5.51	-4.25	-2.98	0.0001

* Study Group 1 = Red meat Phase 1, Chicken-fish Phase 2; Study Group 2 = Chicken-fish Phase 1, red meat Phase 2.

† Estimated treatment effect: Study Group 1 - Study Group 2; Negative result indicates that the value on the red meat diet is higher than the value on the chicken-fish diet.

\# Kilojoules

§ Percentage of total energy intake

¶ Polyunsaturated to saturated fatty acid ratio

** Keys dietary score (Shekelle *et al.*, 1981)

Table 4.20. Direct treatment effect between red meat diet and chicken-fish diet: dietary fatty acids (n = 37)*

Fatty acids (gram)	Lower confidence limit	Difference between means†	Upper confidence limit	p-value
C4:0	-0.035	-0.014	0.006	0.1692
C6:0	-0.021	-0.009	0.004	0.1693
C8:0	-0.016	-0.007	0.002	0.1280
C10:0	-0.036	-0.017	0.002	0.0734
C12:0	-0.077	-0.032	0.012	0.1501
C14:0	-0.349	-0.262	-0.175	0.0001
C14:1	-0.076	-0.065	-0.053	0.0001
C16:0	-1.844	-1.391	-0.938	0.0001
C16:1	-0.227	-0.180	-0.133	0.0001
C18:0	-1.231	-0.982	-0.733	0.0001
C18:1	-3.347	-2.443	-1.540	0.0001
C18:2n-6	-0.465	0.300	1.065	0.4308
C18:3n-3	-0.115	-0.077	-0.038	0.0003
C18:4	0.004	0.006	0.008	0.0001
C20:0	-0.006	0.002	0.009	0.6991
C20:1	0.022	0.031	0.041	0.0001
C20:3	<0.001	0.005	0.009	0.0380
C20:4n-6	-0.002	0.006	0.014	0.1502
C20:5n-3	0.035	0.052	0.068	0.0001
C22:0	-0.008	0.005	0.019	0.4246
C22:1	-0.077	0.015	0.107	0.7450
C22:5n-3	0.016	0.020	0.024	0.0001
C22:6n-3	0.080	0.117	0.155	0.0001
C24:0	-0.004	0.002	0.007	0.5361

* Study Group 1 = Red meat Phase 1, Chicken-fish Phase 2; Study Group 2 = Chicken-fish Phase 1, red meat Phase 2.

† Estimated treatment effect: Study Group 1 - Study Group 2; Negative result indicates that the value on the red meat diet is higher than the value on the chicken-fish diet.

Table 4.21. Estimated differences between the changes on the red meat diet and the reference diet: energy and macronutrient intake, P/S ratio and Keys dietary score

Dietary variables	Lower Confidence limit	Difference between means*	Upper confidence limit	p-value
Energy (kJ)†	-1 904.0	-959.0	-14.0	0.0468
Protein (%E)#	-0.3	1.2	2.9	0.1851
Carbohydrate (%E)	1.5	6.4	11.4	0.0120
Fibre (g)	-1.9	2.5	6.9	0.2668
Total fat (%E)	-11.5	-7.6	-3.7	0.0003
Saturated fat (%E)	-6.2	-4.2	-2.2	0.0001
Monounsaturated fat (%E)	-4.7	-3.0	-1.4	0.0005
Polyunsaturated fat (%E)	-1.5	0.1	1.6	0.9349
P/S ratio§	0.07	0.27	0.46	0.0080
Dietary cholesterol (mg)	-151.9	-93.1	-34.3	0.0026
Keys dietary score¶	-18.10	-11.67	-5.23	0.0007
Alcohol (%E)	-1.46	0.01	1.48	0.9934

* A negative result indicates an increase or decrease over time in the reference group and a decrease in the treatment group; A positive result indicates an increase or decrease over time in the reference group and an increase in the treatment group.

† Kilojoules

Percentage of total energy intake

§ Polyunsaturated to saturated fatty acid ratio

¶ Keys dietary score (Shekelle *et al.*, 1981)

Table 4.22. Estimated differences between the changes on the chicken-fish diet and the reference diet: energy and macronutrient intake, P/S ratio and Keys dietary score

Dietary variables	Lower Confidence limit	Difference between means*	Upper confidence limit	p-value
Energy (kJ)†	-2 464.0	-1 402.0	-342.0	0.0106
Protein (%E)#	-0.2	2.0	3.9	0.0316
Carbohydrate (%E)	5.4	9.2	13.0	0.0001
Fibre (g)	-0.01	3.8	7.6	0.0508
Total fat (%E)	-13.7	-10.8	-7.9	0.0001
Saturated fat (%E)	-7.6	-6.3	-5.0	0.0001
Monounsaturated fat (%E)	-6.9	-5.5	-4.1	0.0005
Polyunsaturated fat (%E)	-0.2	1.2	2.6	0.0874
P/S ratio§	0.59	0.81	1.03	0.0001
Dietary cholesterol (mg)	-151.6	-106.0	-60.5	0.0001
Keys dietary score¶	-24.10	-18.68	-13.25	0.0001
Alcohol (%E)	-1.48	-0.13	1.22	0.8460

* A negative result indicates an increase or decrease over time in the reference group and a decrease in the treatment group; A positive result indicates an increase or decrease over time in the reference group and an increase in the treatment group.
† Kilojoules
# Percentage of total energy intake
§ P/S ratio = polyunsaturated to saturated fatty acid ratio
¶ Keys dietary score (Shekelle *et al.*, 1981)

Table 4.23. Estimated differences between the changes on the red meat diet and the reference diet: dietary fatty acids

Fatty acids (gram)	Lower confidence limit	Difference between means*	Upper confidence limit	p-value
C4:0	-0.365	-0.184	-0.004	0.0459
C6:0	-0.199	-0.096	0.007	0.0663
C8:0	-0.163	-0.058	0.047	0.2699
C10:0	-0.294	-0.125	0.044	0.1429
C12:0	-1.086	-0.291	0.505	0.4663
C14:0	-1.832	-1.007	-0.183	0.0177
C14:1	-0.138	-0.052	0.035	0.2386
C16:0	-7.695	-4.528	-1.360	0.0060
C16:1	-0.900	-0.516	-0.132	0.0096
C18:0	-4.213	-2.276	-0.339	0.0223
C18:1	-13.175	-7.284	-1.394	0.0164
C18:2n-6	-1.420	1.737	4.894	0.2742
C18:3	-0.348	-0.131	0.087	0.2328
C18:4	-0.014	-0.006	0.001	0.1096
C20:0	-0.054	-0.012	0.029	0.5581
C20:1	-0.064	-0.012	0.041	0.6606
C20:3	-0.056	-0.023	0.009	0.1532
C20:4n-6	-0.032	0.006	0.044	0.7536
C20:5n-3	-0.075	-0.033	0.009	0.1241
C22:0	-0.053	0.008	0.068	0.8036
C22:1	-1.094	-0.306	0.481	0.4382
C22:5n-3	-0.024	-0.010	0.003	0.1266
C22:6n-3	-0.139	-0.063	0.013	0.1038
C24:0	-0.017	0.007	0.030	0.5782

* A negative result indicates an increase or decrease over time in the reference group and a decrease in the treatment group; A positive result indicates an increase or decrease over time in the reference group and an increase in the treatment group.

Table 4.24. Estimated differences between the changes on the chicken-fish diet and the reference diet: dietary fatty acids

Fatty acids (gram)	Lower confidence limit	Difference between means*	Upper confidence limit	p-value
C4:0	-0.325	-0.196	-0.067	0.0037
C6:0	-0.177	-0.103	-0.030	0.0071
C8:0	-0.110	-0.048	0.013	0.1213
C10:0	-0.236	-0.126	-0.016	0.0261
C12:0	-0.398	-0.133	0.131	0.3164
C14:0	-1.829	-1.327	-0.825	0.0001
C14:1	-0.138	-0.052	0.035	0.0001
C16:0	-9.313	-6.830	-4.346	0.0001
C16:1	-1.156	-0.796	-0.436	0.0001
C18:0	-5.098	-3.843	-2.588	0.0001
C18:1	-17.369	-11.904	-6.439	0.0001
C18:2n-6	-3.486	0.552	4.590	0.7847
C18:3	-0.417	-0.245	-0.072	0.0065
C18:4	-0.002	0.008	0.016	0.0997
C20:0	-0.097	-0.030	0.036	0.3633
C20:1	-0.020	0.040	0.100	0.1840
C20:3	-0.037	-0.007	0.023	0.6317
C20:4n-6	-0.028	0.021	0.070	0.3936
C20:5n-3	-0.001	0.068	0.137	0.0516
C22:0	-0.142	-0.025	0.091	0.6660
C22:1	-0.318	-0.028	0.263	0.8501
C22:5n-3	0.013	0.031	0.049	0.0010
C22:6n-3	0.021	0.167	0.313	0.0255
C24:0	-0.051	-0.007	0.038	0.7727

* A negative result indicates an increase or decrease over time in the reference group and a decrease in the treatment group; A positive result indicates an increase or decrease over time in the reference group and an increase in the treatment group.

4.3 Discussion

In this study the effect of two prudent diets, differing only in the type of "meat" (lean red meat versus chicken and fish), was tested on plasma lipids, lipoproteins and fatty acid composition of people with elevated plasma cholesterol levels. Dietary compliance is usually of great importance when the effect of different dietary variables on health markers is tested. Special attention was therefore given to the planning and execution of the treatment diets.

There were indications that the dietary energy prescribed during the treatment diets was higher than required. Energy intakes during the baseline periods (seven-day dietary records) were lower than those prescribed, but the difference was only significant in Phase 2 (Table 4.1). The energy intakes on the treatment diets were lower than the energy prescribed and were also lower than the energy intake in the baseline period. In their study on free-living adults in which the effect of three common vegetable oils (in reduced fat diets) on plasma lipids was tested, Insull *et al.* (1994) also found that the energy intake on the test diets was lower than on the *ad libitum* diets. Energy prescribed for the treatment periods could not be based on energy intake determined by means of the seven-day dietary records kept during the baseline period of Phase 1, but had to be calculated theoretically (see Chapter 3). The main reasons for this were the study design and time constraints in terms of coding, computerization and analysis of baseline dietary data before the treatment periods started.

Subjective evaluation by the subjects themselves indicated that compliance with the prescription for the meat exchanges was good (Figure 4.1) and results from the seven-day dietary records confirmed this (Tables 4.4 and 4.5). Results showed only a small difference between the mean portion of lean red meat consumed and the mean portion of chicken plus fish consumed (Table 4.3). Only in Study Group 1 did the number of lean red meat and egg exchanges consumed during the second week of the treatment period of Phase 1 differ significantly from the number of exchanges prescribed.

Subjects indicated that they complied better with the dietary prescriptions in Phase 1 than in Phase 2 which could be symptomatic of respondent burnout as a result of the time span of the study. There were indications that Study Group 1 complied better with the dietary prescriptions than Study Group 2. Results from the seven-day dietary records also showed that Study

Group 2 did not meet the prudent dietary guideline of less than 30%E for fat (Diet Consensus Panel, 1989) on the RMD in Phase 2. However, the remainder of the RMD of Study Group 2 in Phase 2, i.e. saturated fat and dietary cholesterol intake, met the requirements for a prudent diet. Since there is evidence that the subjects complied better with the dietary prescriptions for the CFD than for the RMD (Table 4.6) this could have concealed significant differences between the effect of the two lipid-lowering diets. Results also showed that the subjects found it difficult to consume red meat seven days per week and they violated the dietary prescriptions more often on the RMD than on the CFD. However, the majority of non-compliers violated the dietary prescription for lean red meat or chicken and fish only once or twice during the six weeks of treatment. In a study done by Crumb-Johnson *et al.* (1993), to assess the difference between compliers and non-compliers, results showed that the majority of non-compliers reported one to two episodes of not eating only the food and drink prescribed. It was found that non-compliance was not related to the length of the study (Crumb-Johnson *et al.*, 1993).

During the baseline period the subjects followed a Western type diet with a total fat intake of more than 30%E and a saturated fat intake of more than 10%E. Total fat intakes (37.6 - 38.2%E) of the two treatment groups were higher during the baseline periods of Phase 1 than was previously reported (35.1 - 35.9%E) in another South African study on men and women, 23 to 50 years of age (Wolmarans *et al.*, 1991). Mean cholesterol intakes were not exceptionally high and varied between 275 and 316 mg per day which is in line with the recommendations for a prudent diet.

Comparison of the RMD and CFD with the baseline diets showed that energy, total fat, monounsaturated fat and cholesterol intake were lower, and carbohydrate and protein intake as well as the P/S ratio of the diet were higher on the treatment diets than on the baseline diet. Fibre intake was also higher on the CFD than on the baseline diets. However, fibre intake did not differ between the two treatment diets. The aim to change the Western type diet to a diet which meets the prudent dietary guidelines (Diet Consensus Panel, 1989), was achieved with the treatment diets. With the exception of total fat intake by Study Group 2 on the RMD in Phase 2, the two treatment groups followed a prudent diet in the treatment periods. This showed that, with specialised intervention, it is possible to change the Western type diet followed by many South Africans to a prudent diet.

In the Western Electric Study the diet score of Keys calculated at baseline was positively related to the 19-year follow-up risk of death from CHD (Shekelle *et al.*, 1981). The Keys dietary score is based on dietary saturated fat, polyunsaturated fat and cholesterol intake related to energy intake (Shekelle *et al.*, 1981). This diet score was criticized since in the original Keys equation all the terms in the formula were preceded by Δ, signifying change related to energy intake, fat percentage of calories, or cholesterol (mg) per 1000 kcal (Anon, 1988). It was argued that there is no justification for using this formula to determine the absolute hypercholesterolaemic effect of a diet (Anon, 1988). Nevertheless, the Keys dietary scores for the two treatment diets, RMD and CFD, were calculated to see whether they differed despite the fact that both met the guidelines for a prudent diet. Based on prudent dietary guidelines the calculated Keys dietary score is 28 (Wolmarans *et al.*, 1988) and in the baseline periods the Keys dietary scores were much higher than this figure, indicating that the subjects were following a Western type diet. In the treatment period of Phase 2 the Keys dietary score for Study Group 2 on the RMD was slightly higher than this guideline while the Keys scores on the CFD were much lower than the guideline of 28 which could indicate that the CFD is less atherogenic than the RMD.

Results from the seven-day dietary records showed that both treatment diets met prudent dietary guidelines but significant differences were observed between the RMD and the CFD. The lower intake of energy on the CFD than on the RMD could be due to the lower energy values of chicken and fish than red meat (Gouws & Langenhoven, 1986a). The P/S ratio of chicken (0.84), lean fish (1.93) and fatty fish (1.3) differs from that of red meat (0.1) (Gouws & Langenhoven, 1986a). This difference was reflected in the higher P/S ratio of the CFD than of the RMD. PUFAs have a cholesterol-lowering effect (Insull *et al.*, 1994) and in order to lower plasma cholesterol levels it is recommended that saturated fat intake should be decreased to less than 10%E, and polyunsaturated fat should present 7-10% of energy intake (Adult Treatment Panel II, 1994). One way of lowering SFA intake and replacing it by PUFAs in order to increase the P/S ratio of the diet, is to substitute the fat of red meat in the diet with fat from chicken and fish.

Myristic and palmitic acids are the SFAs responsible for the elevation of plasma cholesterol (Katan, Zock & Mensink, 1994) and both were higher on the RMD than on the CFD. The intake of stearic acid, which does not have a cholesterol-elevating effect (Bonanome & Grundy,

1988), was also higher on the RMD than on the CFD. Fish or marine oils are rich sources of EPA (Kinsella, Lokesh & Stone 1990) and in the Western type diet fish is the main source of EPA. Chicken also contains a small amount of EPA, probably as a result of fish meal which often forms part of the feed of chickens. Since the intake of fish and chicken was not allowed during the RMD the intake of EPA on the RMD should have been zero. Some of the respondents, however, violated the dietary prescription which was reflected in the very low intake of less than 0.01 g EPA per day. In this study the effect of the intake of EPA on the fatty acid composition of plasma CE and TAG (see Chapter 5) served as biomarker for dietary compliance on the RMD.

Although there were significant differences in the intake of energy, fat and cholesterol between the two treatment diets, no significant differences were observed for alcohol, protein and fibre.

The reference group followed a diet with a total fat content which varied between 31.8 and 34.5%E during the study. Their intake at baseline, however, was lower (Table 4.11) than that of the treatment groups (Table 4.9: 37.6 and 35.4%E, Study Group 1; and Table 4.10 : 38.2 and 35.4%E, Study Group 2) which might indicate that they were generally more health conscious in terms of diet than the other groups. Although the reference group knew that they had hypercholesterolaemia they did not receive any lipid-lowering dietary guidelines and they were instructed not to change their habitual diet. The mean percentage of energy derived from total fat remained approximately the same ($\pm$34%E) in the diets of the reference group with the exception of the baseline period of Phase 2 when the percentage of energy from fat decreased to 31.8%E. Thus, despite the fact that the reference group knew they had hypercholesterolaemia, they did not follow a prudent diet during the study. This indicated that the reference group followed the instruction of not to change their habitual diet. It does, however, also demonstrate that people with hypercholesterolaemia do not merely change to a prudent diet because of their condition. Special intervention strategies are required to bring about change in dietary habits. Since everybody in the reference group knew that they had elevated plasma cholesterol levels before the first dietary record was kept, it is possible that they could have changed their dietary intake unconsciously throughout the study. This demonstrated the importance of a baseline period in intervention studies to compensate for the Hawthorne (also called placebo) effect. The reference group did, however, differ from the treatment groups as was shown by the significant differences observed between the changes on the RMD and the

reference diet or the CFD and the reference diet.

Although it was previously shown in a cross-sectional study on South Africans who followed a Western type diet that the main source of total fat, saturated fat and dietary cholesterol was red meat (Wolmarans *et al.*, 1988), only 5.3% of the subjects in the present study indicated that they would prefer to eat red meat five times per week. The preference of red meat by South Africans who follow a Western type diet might therefore be less than is generally believed.

4.4 Conclusions

* It could be concluded that compliance with the dietary prescription was good. Non-compliance probably could not have influenced the outcome of this study, because the non-compliant subjects only transgressed the dietary prescriptions a few times.

* This study showed that it is possible to change a Western type diet of free-living subjects to a prudent diet within six weeks. Specialised and intensive dietary intervention, however, is needed in order to achieve this goal in the long-term.

* Cross-over analysis showed no significant differences for the baseline comparisons and the direct-by-period interaction effect for energy, macronutrient and fatty acid intakes. This indicated that there were no first-order or second-order carry-over effects. The measurements of the second treatment period could thus be used in the estimation of the direct treatment effects.

* The Keys dietary scores were higher on the prudent RMD than on the prudent CFD which might indicate that the latter is less atherogenic. The fatty acid profile of the diet also confirms this observation since myristic and palmitic acids, the SFAs responsible for the elevation of plasma cholesterol, were significantly higher on the RMD than on the CFD.

* Evidence from this study shows that the preference of red meat among South Africans who follow a Western type diet is less than is generally believed.

CHAPTER 5

THE INFLUENCE OF DIETARY INTERVENTION ON PLASMA LIPIDS AND LIPOPROTEINS AND BLOOD PRESSURE OF SUBJECTS WITH ELEVATED PLASMA TOTAL CHOLESTEROL LEVELS

5.1 Introduction

Hypercholesterolaemia is one of the major risk factors responsible for the development of CHD. LDL-C is regarded as the most atherogenic lipoprotein. A plasma TC level of $\leq$5.2 mmol/L and an LDL-C level of $\leq$3.4 mmol/L are regarded as desirable (Adult Treatment Panel II, 1994). It has been shown that more than 50% of white South Africans had a serum TC level of $\geq$5.7 mmol/L (Rossouw *et al.*, 1983). In 1988 age-specific cut-off points were published for South Africans (Rossouw *et al.*, 1988). Steyn *et al.* (1987) showed that 69.2% of coloured South African men and 65.9% of coloured women were at risk for CHD by virtue of their TC level. A high prevalence of hypercholesterolaemia was also found in Indian South Africans (Seedat *et al.*, 1990) while the prevalence of hypercholesterolaemia is still low in black South Africans (Steyn *et al.*, 1991).

The independent effect of TAG on the development of CHD has not yet been confirmed (Austin *et al.*, 1991). It is not clear what role the metabolic interrelationship between HDL-C and TAG plays in the relationship between TAG and CHD (Austin *et al.*, 1991). Patch *et al.* (1992) showed that fasting TAG levels are not an independent risk factor for CHD while postprandial TAG levels are. There is a strong inverse relationship between HDL-C and CHD (Ginsberg, 1990) and disturbances in the metabolism of TAG-rich particles may explain the low HDL-C levels found in CHD (Assmann, Von Eckardstein & Funke, 1993).

Dietary intervention is always the first step in the treatment of hypercholesterolaemia (Adult Treatment Panel II, 1994). The main characteristics of the diet for the prevention of CHD is the lowering of total fat intake, especially saturated fat. The lowering of total fat intake will usually result in an increased intake of carbohydrate. A total fat intake of 20%E (American

Heart Association, 1984) or even 10%E (Ornish *et al.*, 1990) is favoured by some in the treatment of those with severely elevated plasma TC levels. There are, however, indications that a low fat, high carbohydrate diet tends to decrease HDL-C (Morgan, Sinclair & O'Dea, 1993; Grundy, 1986) and increase TAG (Morgan, Sinclair & O'Dea, 1993; Sanders *et al.*, 1994; Grundy, 1986). Epidemiological studies indicate that HDL-C has a protective effect (American Heart Association, 1982) but it is not clear whether a decrease in HDL-C by diet would influence the risk of CHD negatively (Katan, Zock & Mensink, 1995). When fat in the diet is replaced by carbohydrate TAG increases (Katan, Zock & Mensink, 1995), although not always (Grundy *et al.*, 1988b). Although very low fat diets seem to increase TAG, moderate carbohydrate intakes, ≤60%E, do not seem to have a deleterious effect on TAG concentrations (Retzlaff, 1995). Restriction of saturated and not total fat intake (Morgan, Sinclair & O'Dea, 1993) seems to be the most important dietary recommendation for the lowering of TC and LDL-C. The SFAs which are responsible for the elevation of TC are lauric, myristic and palmitic acids (Keys, Anderson & Grande, 1965b; Hegsted *et al.*, 1965; Denke & Grundy, 1992; Zock, De Vries & Mensink, 1994). Palmitic acid is the SFA that predominates in the Western type diet (Katan, Zock & Mensink, 1995). Dietary cholesterol should also be restricted in a cholesterol-lowering diet (Grundy *et al.*, 1988a; Shekelle & Stamler, 1989; Adult Treatment Panel II, 1994) although there is still some controversy about its effect on plasma TC levels in humans (Grundy & Denke, 1990).

Nowadays it is recommended that total fat should be ≤30%E. For those with hypercholesterolaemia a saturated fat intake of ≤7%E is prescribed, compared to 8-10%E recommended for the general population (Adult Treatment Panel II, 1994).

The recommendation that saturated fat intake should be reduced implies that the intake of animal fat, which is known to be high in saturated fat, should be restricted. This has implications for the intake of foods such as red meat, milk, and milk products. In an effort to lower TC concentrations meatless meals (no fish, poultry, red meat, eggs, cheese >3% fat) were introduced for hypercholesterolaemic subjects in the MRFIT study. Serum TC levels were lowered by 2.6% (Daniel-Gentry *et al.*, 1986). Red meat is usually implicated as a major source of SFAs and dietary cholesterol which are both associated with the elevation of plasma TC concentrations (Keys, Anderson & Grande, 1965b; Hegsted *et al.*, 1965; Grundy & Denke, 1990; Denke & Grundy, 1992; Zock, De Vries & Katan, 1994). Red meat makes a substantial

contribution to the total fat, saturated fat and dietary cholesterol intake of white South Africans (Wolmarans *et al.*, 1989) known to have a high prevalence of hypercholesterolaemia (Rossouw *et al.*, 1983). By studying the effect of lean red meat on plasma cholesterol, O'Dea *et al.* (1990) showed that it was the fat and not the meat itself that had a cholesterol elevating effect. There are also indications that beef that is highly marbled may not contribute to a higher cholesterol intake than beef with little marbling (Sweeten *et al.*, 1990).

The New Zealand dietary guidelines for hypercholesterolaemics not only recommend the lowering of total fat and saturated fat intake, but give specific recommendations in connection with the consumption of red meat (Mann *et al.*, 1993). It is recommended that red meat intake should be limited to less than 150-200 g cooked weight per day for men and 100-150 g per day for women. This is in agreement with the recommendation of $\leq$5 to 6 oz per day by the NCEP (Adult Treatment Panel II, 1994).

In order to formulate recommendations for red meat consumption for hyperlipidaemics in South Africa, the effect of two prudent diets, which differed only in the type of "meat" (lean red meat versus chicken, without skin, and fish) on plasma lipids and lipoproteins of free-living subjects with age-related elevated plasma cholesterol levels were tested. The study also investigated whether the change in plasma TC, as a result of the treatment diets, could be predicted by the Keys equation (Keys, Anderson & Grande, 1965b).

In this chapter results on the family history and of risk factors for CHD will be given. The effect of the test diets on body mass, plasma lipid and lipoprotein concentrations and blood pressure will also be reported.

5.2 Results

Complete biochemical and anthropometric data on 52 subjects were collected. Reasons why 18 (25%) of the respondents did not complete the study are given in Chapter 4.

5.2.1 Risk factor questionnaire

Information collected by means of a questionnaire (Addendum B) showed that some of the subjects had a family history of CHD, hypercholesterolaemia, high blood pressure and stroke. Some of the subjects also had a family history of diabetes mellitus (Table 5.1).

Table 5.1. Number of subjects with a family history of coronary heart disease, high blood pressure and diabetes mellitus

	Study Groups		
	1 (n =19)	2 (n = 18)	3 (n = 13)
	Number of subjects		
Coronary heart disease (CHD)			
Father	7	7	3
Mother	3	3	0
CHD before the age of 50 years			
Father	2	3	0
Mother	1	2	0
High blood cholesterol			
Father	4	4	2
Mother	1	4	1
High blood pressure			
Father	4	2	3
Mother	5	4	3
Stroke			
Father	5	2	2
Mother	0	1	0
Diabetes mellitus			
Father	1	0	1
Mother	2	3	1

Hypercholesterolaemia seemed to be more prevalent in the family members of Study Group 2 than in those of Study Group 1 and Study Group 3. Seven of the subjects smoked; two in Study Group 1, one in Study Group 2 and four in Study Group 3. They smoked between 6 and 30 cigarettes per day. The majority of the subjects were inactive during their workday while after

hours only four of Study Group 1, six of Study Group 2 and three of Study Group 3 were very active. Sixteen (84.2%) of the subjects in Study Group 1, thirteen (72.2%) in Study Group 2 and 10 (76.9%) in Study Group 3 consumed alcohol. Only five subjects received a cholesterol-lowering diet previously and only three indicated that they followed the diet.

5.2.2 Effects of test diets on body mass, plasma lipids and lipoproteins and blood pressure

5.2.2.1 Mean values

In Tables 5.2, 5.3 and 5.4 mean values and standard deviations for body mass, plasma lipids and lipoproteins, and blood pressure for the baseline and treatment periods are given for the different groups.

5.2.2.2 Baseline versus treatment periods

In Table 5.5 differences in body mass, plasma lipoproteins, blood pressure and uric acid levels between the baseline and the treatment periods are given. Several significant differences between the baseline and the treatment periods were found.

5.2.2.3 Cross-over analysis

Except for LDL_2-apo B ($p=0.0365$), there were no differences in the baseline values of Study Group 1, Study Group 2 and Study Group 3. Study Group 1 and Study Group 2 (the treatment diets) did not differ for LDL_2-apo B but Study Group 2 and Study Group 3 differed ($p=0.0365$) and it was higher for Study Group 2 than for Study Group 3 (difference between means was 4.3 mg/dL). Except for VLDL-TAG ($p=0.0336$), there were no significant differences in baseline values between Study Group 1 and Study Group 2.

In Table 5.6 the p-values for the differences between the baseline comparisons (carry-over effect) and the direct-by-period interaction effect are given. There is a significant carry-over effect (baseline comparisons) for VLDL-TAG but no other significant differences for the baseline comparisons or for the direct-by-period interaction effect were observed.

Table 5.2. Mean (and standard deviation) body mass, plasma lipids and lipoproteins, and blood pressure of subjects in the baseline and treatment periods (Study Group 1, n = 21)

Variables		Diets			
		Red meat		Chicken-fish	
		B_1*	T_1†	B_2#	T_2§
Body Mass (kg)	Mean	74.4	73.1	74.4	73.3
	SD¶	11.6	10.6	11.1	10.9
Total cholesterol (mmol/L)	Mean	5.58	5.18	5.52	5.11
	SD	1.02	1.03	1.19	1.04
Triacylglycerol (mmol/L)	Mean	1.02	1.11	1.16	1.11
	SD	0.42	0.29	0.35	0.32
HDL-cholesterol** (mmol/L)	Mean	1.34	1.32	1.30	1.26
	SD	0.31	0.37	0.34	0.41
HDL_2-C (mmol/L)	Mean	0.25	0.17	0.16	0.20
	SD	0.19	0.18	0.19	0.26
HDL_3-C (mmol/L)	Mean	1.10	0.15	1.14	1.06
	SD	0.15	0.24	0.18	0.18
LDL_1-cholesterol†† (mmol/L)	Mean	0.43	0.31	0.44	0.36
	SD	0.16	0.11	0.23	0.18
LDL_2-cholesterol (mmol/L)	Mean	3.26	2.92	3.22	2.71
	SD	0.86	0.86	0.94	0.67
LDL_1-apo B lipoprotein (mg/dL)	Mean	7.19	5.01	6.14	5.75
	SD	2.11	2.10	2.77	2.75
LDL_2-apo B lipoprotein (mg/dL)	Mean	60.84	51.92	67.69	55.87
	SD	17.93	17.28	21.50	18.27
VLDL-cholesterol## (mmol/L)	Mean	0.51	0.53	0.49	0.53
	SD	0.34	0.28	0.22	0.22
VLDL-TAG (mmol/L)	Mean	0.57	0.67	0.63	0.57
	SD	0.29	0.20	0.25	0.21
Systolic blood pressure (mm Hg)	Mean	120.7	121.6	118.7	116.9
	SD	10.6	10.2	9.7	10.3
Diastolic blood pressure (mm Hg)	Mean	76.2	78.8	73.0	74.1
	SD	7.2	8.6	10.5	9.3

* Baseline period of Phase 1
† Week six of treatment period of Phase 1
# Baseline period of Phase 2
§ Treatment period of Phase 2
¶ Standard deviation
** High density lipoprotein cholesterol
†† Low density lipoprotein cholesterol
Very low density lipoprotein cholesterol

Table 5.3. Mean (and standard deviation) body mass, plasma lipids and lipoproteins, and blood pressure of subjects in the baseline and treatment periods (Study Group 2, n =18)

Variables		Diets			
		Chicken-fish		Red meat	
		B_1*	T_1†	B_2#	T_2§
Body Mass (kg)	Mean	72.5	71.0	71.3	71.4
	SD¶	12.3	11.9	12.1	12.1
Total cholesterol (mmol/L)	Mean	5.43	4.93	5.20	5.05
	SD	1.05	1.07	1.34	1.06
Triacylglycerol (mmol/L)	Mean	1.11	1.00	1.04	1.01
	SD	0.48	0.35	0.48	0.36
HDL-cholesterol** (mmol/L)	Mean	1.33	1.31	1.34	1.38
	SD	0.34	0.34	0.32	0.36
HDL_2-C (mmol/L	Mean	0.25	0.21	0.23	0.25
	SD	0.20	0.17	0.18	0.18
HDL_3-C (mmol/L)	Mean	1.08	1.11	1.11	1.13
	SD	0.18	0.23	0.17	0.20
LDL_1-cholesterol†† (mmol/L)	Mean	0.43	0.34	0.41	0.38
	SD	0.17	0.18	0.21	0.17
LDL_2-cholesterol (mmol/L)	Mean	3.11	2.75	2.92	2.75
	SD	0.94	0.90	1.09	0.96
LDL_1-apo B lipoprotein (mg/dL)	Mean	7.05	5.58	5.56	5.55
	SD	2.26	3.15	2.47	2.35
LDL_2-apo B lipoprotein (mg/dL)	Mean	58.62	47.57	60.19	52.58
	SD	18.11	15.71	22.39	17.62
VLDL-cholesterol## (mmol/L)	Mean	0.49	0.41	0.47	0.45
	SD	0.22	0.16	0.28	0.17
VLDL-TAG (mmol/L)	Mean	0.63	0.59	0.54	0.46
	SD	0.35	0.28	0.34	0.22
Systolic blood pressure (mm Hg)	Mean	115.2	114.7	113.2	113.0
	SD	9.7	7.9	7.1	7.8
Diastolic blood pressure (mm Hg)	Mean	73.7	69.9	70.1	71.8
	SD	9.1	8.5	7.7	9.8

* Baseline period of Phase 1
† Week six of treatment period of Phase 1
# Baseline period of Phase 2
§ Treatment period of Phase 2
¶ Standard deviation
** High density lipoprotein cholesterol
†† Low density lipoprotein cholesterol
Very low density lipoprotein cholesterol

Table 5.4. Mean (and standard deviation) body mass, plasma lipids and lipoproteins, and blood pressure of subjects in the baseline and treatment periods (Study Group 3, n = 13)

		Diets			
		Habitual		Habitual	
Variables		B_1*	T_1†	B_2#	T_2§
Body Mass (kg)	Mean	73.6	73.6	73.8	73.6
	SD¶	11.9	11.7	11.7	12.0
Total cholesterol (mmol/L)	Mean	5.49	5.48	5.50	5.17
	SD	1.14	1.26	1.08	1.10
Triacylglycerol (mmol/L)	Mean	1.32	1.35	1.48	1.60
	SD	0.68	0.70	0.75	1.19
HDL-cholesterol** (mmol/L)	Mean	1.34	1.32	1.33	1.28
	SD	0.36	0.34	0.35	0.37
HDL_2-C (mmol/L)	Mean	0.22	0.18	0.15	0.19
	SD	0.16	0.13	0.14	0.17
HDL_3-C (mmol/L)	Mean	1.11	1.14	1.18	1.09
	SD	0.23	0.23	0.26	0.25
LDL_1-cholesterol†† (mmol/L)	Mean	0.48	0.44	0.50	0.38
	SD	0.23	0.18	0.22	0.18
LDL_2-cholesterol (mmol/L)	Mean	2.94	3.01	2.94	2.67
	SD	0.95	1.05	0.92	0.86
LDL_1-apo B lipoprotein (mg/dL)	Mean	7.88	7.98	7.40	5.91
	SD	3.15	3.55	2.71	2.47
LDL_2-apo B lipoprotein (mg/dL)	Mean	53.54	54.12	63.58	54.47
	SD	14.85	20.13	19.32	15.82
VLDL-cholesterol## (mmol/L)	Mean	0.62	0.60	0.66	0.73
	SD	0.35	0.42	0.41	0.50
VLDL-TAG (mmol/L)	Mean	0.80	0.84	0.86	0.86
	SD	0.53	0.54	0.56	0.80
Systolic blood pressure (mm Hg)	Mean	115.5	116.2	115.7	115.2
	SD	9.2	8.1	8.0	8.7
Diastolic blood pressure (mm Hg)	Mean	73.1	72.5	69.4	72.5
	SD	8.9	10.0	8.7	7.4

* Baseline period of Phase 1
† Week six of treatment period of Phase 1
\# Baseline period of Phase 2
§ Treatment period of Phase 2
¶ Standard deviation
** High density lipoprotein cholesterol
†† Low density lipoprotein cholesterol
Very low density lipoprotein cholesterol

Table 5.5. Difference in body mass, plasma lipids and lipoproteins, and blood pressure between the baseline period and the treatment period (treatment period value - baseline period value)

Variables	Diets					
	Red meat			Chicken-fish		
	Mean n=39	SD*	p-value	Mean n=39	SD	p-value
Body mass (kg)	-0.7	2.0	0.0651	-1.2	1.6	0.0001
TC† (mmol/L)	-0.29	0.63	0.0084	-0.45	0.55	0.0001
HDL-C# (mmol/L)	0.004	0.13	0.8965	-0.03	0.14	0.2354
HDL-C:TC ratio	0.01	0.03	0.0170	0.02	0.04	0.0025
HDL_2-C (mmol/L)	-0.03	0.09	0.0362	0.004	0.11	0.7448
HDL_3-C (mmol/L)	0.04	0.15	0.1371	-0.03	0.13	0.0267
LDL_1-C§ (mmol/L)	-0.08	0.13	0.0003	-0.09	0.14	0.0003
LDL_2-C (mmol/L)	-0.27	0.49	0.0014	-0.44	0.55	0.0001
LDL_1-apo B¶ (mg/dL)	-1.18	2.13	0.0014	-0.89	2.18	0.0133
LDL_2-apo B (mg/dL)	-8.32	10.90	0.0001	-11.46	9.69	0.0001
VLDL-C (mmol/L)**	0.002	0.22	0.8537	-0.02	0.15	0.3111
TAG†† (mmol/L)	0.03	0.39	0.5353	-0.08	0.32	0.3199
VLDL-TAG (mmol/L)	0.02	0.26	0.5370	-0.05	0.23	0.3079
Blood pressure:						
Systolic (mm Hg)	0.44	6.84	0.8935	-1.23	8.59	0.4242
Diastolic (mm Hg)	2.18	5.91	0.0286	-1.18	6.91	0.3489

* Standard deviation
† Total cholesterol
# High density lipoprotein cholesterol
§ Low density lipoprotein cholesterol
¶ LDL-apolipoprotein B
** Very low density lipoprotein cholesterol
†† Triacylglycerol

Table 5.6. P-values for the differences between the baseline comparisons (carry-over effect) and the direct-by-period interaction effect for Study Groups 1 and 2 for body mass, plasma lipids and lipoproteins, and blood pressure (n = 39)

Variables	Baseline comparisons (carry over effect)	Direct-by-period interaction effect
	p-value	p-value
Body mass (kg)	0.1220	0.3822
TC* (mmol/L)	0.3039	0.6140
HDL-C† (mmol/L)	0.3039	0.1452
HDL_2-C (mmol/L)	0.0774	0.6451
HDL_3-C (mmol/L)	0.6086	0.2137
LDL_1-C§ (mmol/L)	0.4390	0.3242
LDL_2-C (mmol/L)	0.2761	0.2199
LDL_1-apo B¶ (mg/dL)	0.4014	0.3146
LDL_2-apo B (mg/dL)	0.0890	0.7017
VLDL-C** (mmol/L)	0.9136	0.1177
TAG†† (mmol/L)	0.0579	0.2582
VLDL-TAG (mmol/L)	0.0336	0.1642
Blood pressure:		
Systolic (mm Hg)	0.9855	0.9702
Diastolic (mm Hg)	0.8404	0.0636

* Total cholesterol
† High density lipoprotein cholesterol
§ Low density lipoprotein cholesterol
¶ LDL-apolipoprotein B
** Very low density lipoprotein cholesterol
†† Triacylglycerol

5.2.2.4 Direct treatment effect

The direct treatment effect refers to the effect of the RMD and the CFD within the cross-over design, on body mass, plasma lipids and lipoproteins, and blood pressure. In Table 5.7 the estimated treatment effect, the lower and upper confidence limits and p values are given for the direct treatment effect of the RMD and the CFD. A negative value indicates that the mean effect on the RMD was higher than on the CFD. There was a significant direct treatment effect for HDL-C ($p=0.0498$) which was higher on the RMD than on the CFD (Table 5.7). The effect, however, was very small (0.04 mmol/L). A significant treatment effect ($p=0.0027$) between the RMD and the CFD was also observed for diastolic blood pressure. Diastolic blood pressure

was significantly lower on the CFD than on the RMD but the difference was small, viz. 1.6 mm Hg. Although no other significant direct treatment effects were observed between the RMD and the CFD, there are indications that, except for LDL_1-C, LDL_1-apo B and VLDL-TAG, variables tended to be higher on the RMD than on the CFD.

Table 5.7. Direct treatment effect between red meat diet and chicken-fish diet: body mass, plasma lipids and lipoproteins, and blood pressure (n =39)*

Variables	Lower confidence limit	Difference between means†	Upper confidence limit	p-value
Body mass (kg)	-0.30	-0.03	0.25	0.8509
TC# (mmol/L)	-0.12	-0.05	0.03	0.2261
HDL-C§ (mmol/L)	-0.06	-0.03	-<0.001	0.0498
HDL_2-C (mmol/L)	-0.02	-0.003	0.02	0.7759
HDL_3-C (mmol/L)	-0.06	-0.03	<0.001	0.0555
LDL_1-C¶ (mmol/L)	-0.02	0.002	0.02	0.8562
LDL_2-C (mmol/L)	-0.13	-0.05	0.03	0.1924
LDL_1-apo B**	-0.13	0.19	0.52	0.2390
LDL_2-apo B	-1.75	-0.26	1.21	0.7193
VLDL-C†† (mmol/L)	-0.04	-0.01	0.02	0.4413
TAG## (mmol/L)	-0.05	-0.002	0.04	0.9088
VLDL-TAG (mmol/L)	-0.02	0.01	0.03	0.4644
Blood pressure:				
Systolic (mm Hg)	-2.06	-0.77	0.51	0.2295
Diastolic (mm Hg)	-2.67	-1.64	-0.61	0.0027

* Study Group 1 = Red meat Phase 1, Chicken-fish Phase 2; Study Group 2 = Chicken-fish Phase 1, red meat Phase 2.

† Estimated treatment effect: Study Group 1 - Study Group 2; Negative result indicates that the value on the red meat diet is higher than the value on the chicken-fish diet.

\# Total cholesterol

§ High density lipoprotein cholesterol

¶ Low density lipoprotein cholesterol

** LDL-apolipoprotein B

†† Very low density lipoprotein cholesterol

Triacylglycerol

5.2.2.5 Treatment diets versus reference diet

In Tables 5.8 and 5.9 the estimated differences between the change on the RMD and the reference diet and between the change on the CFD and the reference diet are given. A negative result indicates an increase over time in the reference group and a decrease over time in the treatment group. The opposite is true of a positive result. There were no significant differences between the RMD and the reference diet (Table 5.8). Body mass, LDL_2-C and LDL_2-apo B, however, were lower on the CFD than on the reference diet. Plasma TC ($p = 0.0927$) was also lower on the CFD than on the reference diet (Table 5.9).

Table 5.8. Estimated differences between the changes on the red meat diet and the reference diet: body mass, plasma lipids and lipoproteins, and blood pressure

Variables	Lower confidence limit	Difference between means*	Upper confidence limit	p-value
Body mass (kg)	-1.68	-0.51	0.66	0.3862
TC† (mmol/L)	-0.49	-0.12	0.25	0.5296
HDL-C# (mmol/L)	-0.05	0.04	0.12	0.3701
HDL_2-C (mmol/L)	-0.08	-0.03	0.02	0.2672
HDL_3-C (mmol/L)	-0.02	0.07	0.15	0.1330
LDL_1-C§ (mmol/L)	-0.08	0.002	0.08	0.9572
LDL_2-C (mmol/L)	-0.45	-0.16	0.13	0.2649
LDL_1-apo B¶ (mg/dL)	-1.80	-0.48	0.84	0.4704
LDL_2-apo B (mg/dL)	-10.82	-4.10	2.63	0.2266
VLDL-C (mmol/L)**	-0.16	-0.02	0.12	0.7403
TAG†† (mmol/L)	-0.29	-0.04	0.22	0.7628
VLDL-TAG (mmol/L)	-0.18	-0.01	0.16	0.9200
Blood pressure:				
Systolic (mm Hg)	-3.67	0.36	4.39	0.8587
Diastolic (mm Hg)	-2.62	0.95	4.51	0.5954

* A negative result indicates an increase over time in the reference group and a decrease in the treatment group; A positive result indicates an increase over time in the reference group and an increase in the treatment group.
† Total cholesterol
# High density lipoprotein cholesterol;
§ Low density lipoprotein cholesterol;
¶ LDL-apolipoprotein B;
** Very low density lipoprotein;
†† Triacylglycerol.

Table 5.9. Estimated differences between the changes on the chicken-fish diet and the reference diet: body mass, plasma lipids and lipoproteins, and blood pressure

Variables	Lower confidence limit	Difference between means*	Upper confidence limit	p-value
Body mass (kg)	-2.03	-1.07	-0.12	0.0280
TC† (mmol/L)	-0.61	-0.28	0.05	0.0927
HDL-C# (mmol/L)	-0.08	0.01	0.09	0.8777
HDL_2-C (mmol/L)	-0.05	0.009	0.07	0.7686
HDL_3-C (mmol/L)	-0.08	-0.002	0.08	0.9507
LDL_1-C§ (mmol/L)	-0.09	-0.01	0.08	0.8980
LDL_2-C (mmol/L)	-0.66	-0.34	0.02	0.0401
LDL_1-apo B¶ (mg/dL)	-1.53	-0.19	1.16	0.7804
LDL_2-apo B (mg/dL)	-13.35	-7.24	-1.13	0.0211
VLDL-C (mmol/L)**	-0.15	-0.04	0.06	0.4145
TAG†† (mmol/L)	-0.37	-0.15	0.07	0.1812
VLDL-TAG (mmol/L)	-0.23	-0.07	0.08	0.3291
Blood pressure:				
Systolic (mm Hg)	-6.28	-1.31	3.67	0.5997
Diastolic (mm Hg)	-6.50	-2.41	1.68	0.2422

* A negative result indicates an increase over time in the reference group and a decrease in the treatment group; A positive result indicates an increase over time in the reference group and an increase in the treatment group.
† Total cholesterol
# High density lipoprotein cholesterol
§ Low density lipoprotein cholesterol
¶ LDL-apolipoprotein B
** Very low density lipoprotein cholesterol
†† Triacylglycerol.

5.2.3 Plasma total cholesterol versus age-dependent action limits for the treatment of hypercholesterolaemia

In Figures 5.1, 5.2, 5.3 and 5.4 plasma TC is plotted against age, and the age-dependent cut off points for low, moderate and high risk in terms of TC, are indicated. At recruitment only one of the 39 subjects did not have moderately or severely elevated plasma TC (Figure 3.1). Results of the baseline period of Phase 1 indicate that at the end of the first week the plasma TC levels of five subjects fell within normal limits (Figure 5.2) while at the end of the third week eight subjects had plasma TC levels within normal limits. Fourteen subjects had normal

plasma TC levels at the end of week two of the baseline periods for Phase 2. Taking the cross-over design into consideration, analysis for the baseline values that preceded the different treatment diets showed that at the end of the three week baseline period for the RMD plasma TC levels of 14 subjects fell within normal limits while for the CFD this figure was nine (Figure 5.3). A marked shift of subjects into the low risk category on both the prudent diet containing lean red meat (Figure 5.3) and the prudent diet containing chicken (without skin) and fish (Figure 5.3) could be observed. Twenty-five (64.1%) subjects had elevated plasma TC before dietary intervention with the RMD and 19 (48.7%) still had hypercholesterolaemia after dietary intervention. Thirty (76.9%) subjects had hypercholesterolaemia before dietary intervention with the CFD and 18 (46.2%) still had hypercholesterolaemia after dietary intervention. A major shift in plasma TC could be observed between the baseline and treatment periods. Plasma TC decreased in 26 (66.7%) of those on the RMD and in 33 (84.6%) of those on the CFD, while plasma TC increased in 13 (33.3%) of those on the RMD and in six (15.4%) of those on the CFD. Figure 5.4 shows the plasma TC levels at the end of the post-treatment period. Results clearly show that there was a shift of plasma TC levels back to elevated levels.

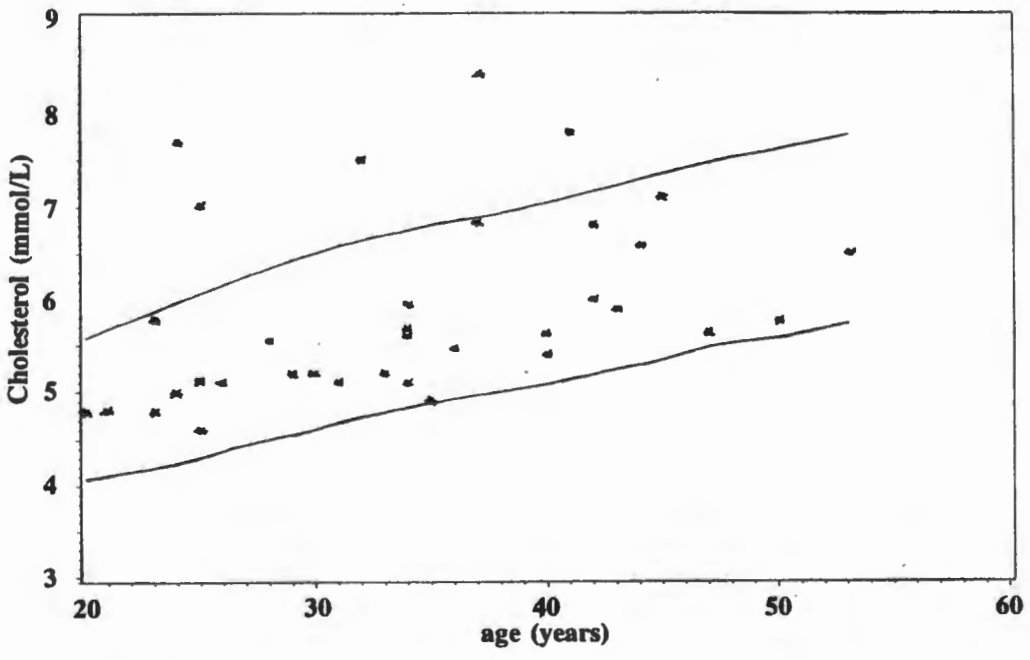

Figure 5.1. Plasma total cholesterol versus age-dependent action limits for the treatment of hypercholesterolaemia - recruitment values at baseline for treatment group (n = 39)

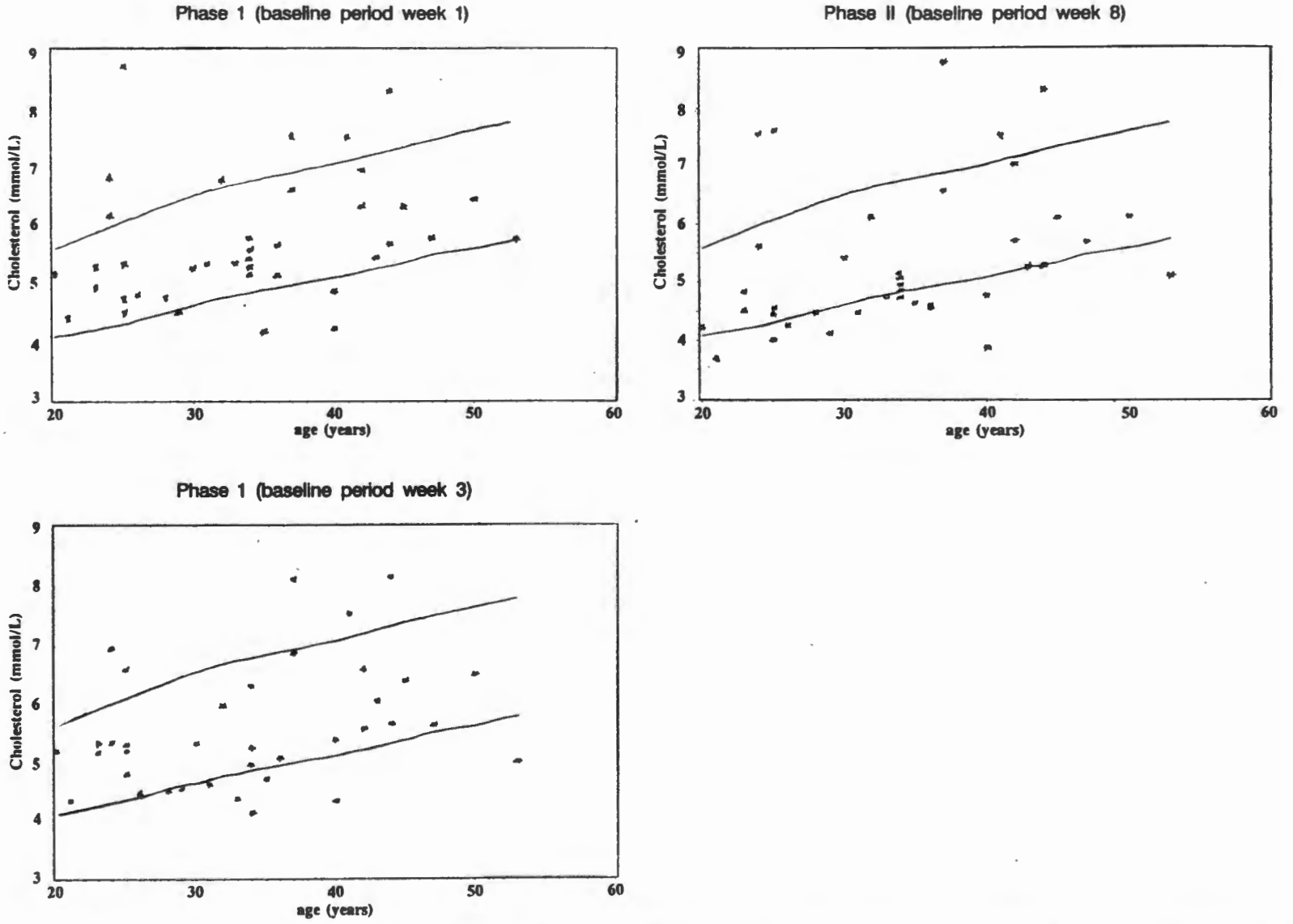

Figure 5.2 Plasma total cholesterol versus age-dependent action limits for the treatment of hypercholesterolaemia for baseline period values for the treatment group (n = 39)

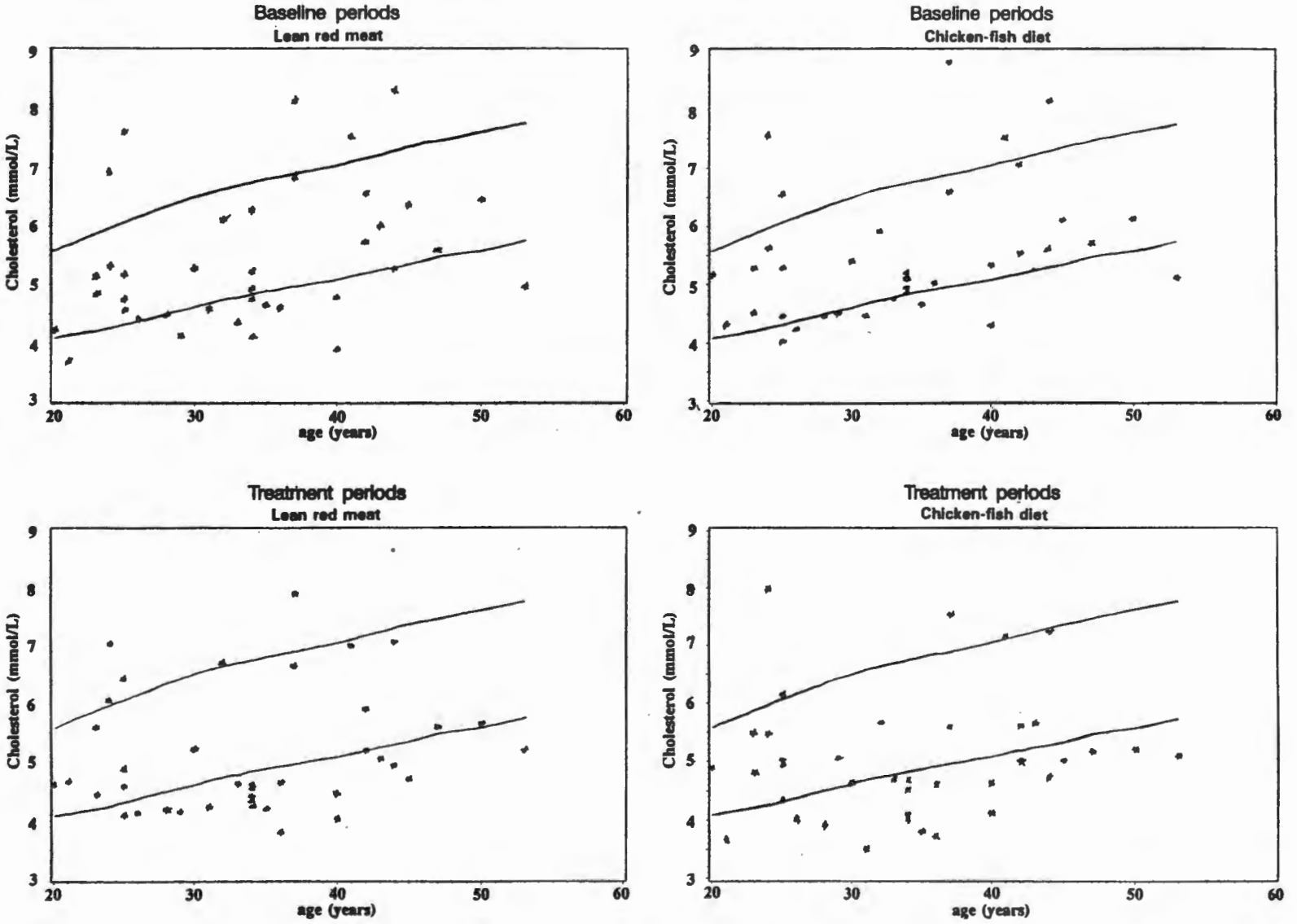

Figure 5.3 **Plasma total cholesterol versus age-dependent action limits for the treatment of hypercholesterolaemia for baseline and treatment period values for the red meat diet and the chicken-fish diet for the treatment group (n = 39)**

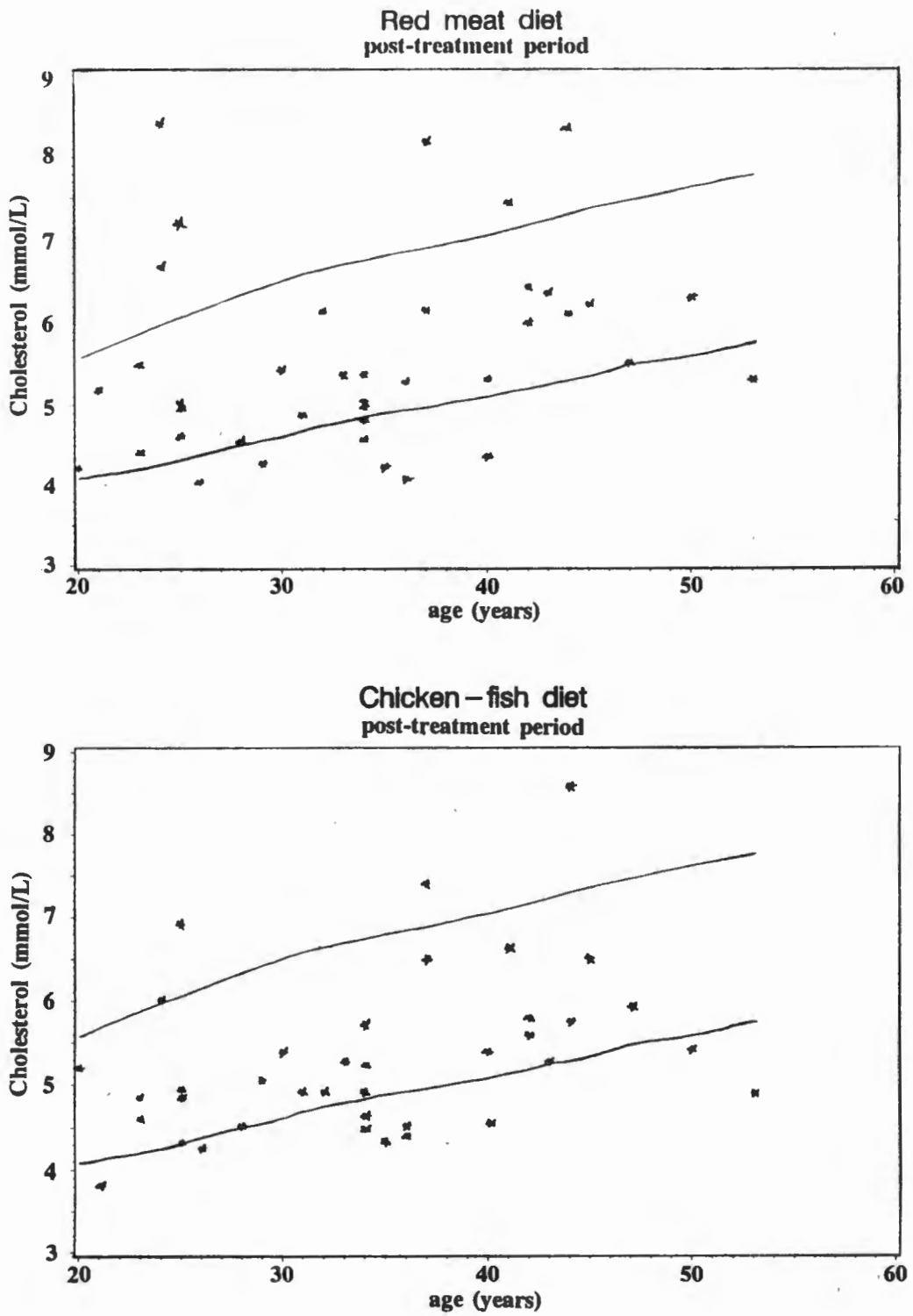

Figure 5.4 **Plasma total cholesterol versus age-dependent action limits for the treatment of hypercholesterolaemia - post-treatment period values for the red meat diet and the chicken-fish diet (n = 39)**

5.2.4 Keys equation

In Table 5.10 the Keys equation (Keys, Anderson & Grande, 1965b) for the estimation of the average change in plasma TC response, to a dietary change in the percentage of energy from SFAs and PUFAS and dietary cholesterol intake is given for different periods: (a) the baseline period for Phase 1 minus the baseline period for Phase 2; (b) the two treatment diets, red meat diet minus chicken-fish diet; (c) the baseline period diet for red meat minus the red meat treatment diet; (d) the baseline period diet for chicken-fish minus the chicken-fish treatment diet. The Keys equation was calculated using different combinations of SFAs and PUFAs: (A) total SFAs and total PUFAs; (B) total SFAs and C18:2; (C) C12:0 + C14:0 + C16:0 and total PUFAs; (D) C12:0 + C14:0 + C16:0 and C18:2; (E) C12:0-C18:0 and total PUFAs; (F) C12:0-C18:0 and C18:2.

The measured differences in plasma TC is also given in Table 5.10 for the different periods. There was good agreement between some of the mean values predicted (Keys equation) and the measured mean change in plasma TC levels. Looking at the mean values the combinations of total SFAs and total PUFAs (A) and total SFAs and linoleic acid (B) used in the Keys equation gave the best fit for the predicted mean change and the measured mean change in TC between the baseline period and the RMD. The measured change in plasma TC between the baseline period and the chicken-fish diet was best predicted by E (C12:0-C18:0 and total PUFAs) and F (C12:0-C18:0 and C18:2).

Mean differences between the predicted change in plasma TC and the measured change in plasma TC are given in Table 5.11. Only when total SFAs and total PUFAs or C12:0 + C14:0 + C16:0 and total PUFAs were used in the Keys equation did the predicted change and measured change in plasma TC between the baseline and CFD differed significantly.

Table 5.10. Prediction of change in plasma total cholesterol (mmol/L) level (Keys equation) and measured change in plasma total cholesterol (n = 37)

	Diet			
Keys equation*	**B[a]**	**T[b]**	**RMD[c]**	**CFD[d]**
A (SFA†/PUFA#)				
mean	0.03	0.26	0.30	0.52
SD§	0.33	0.34	0.41	0.35
B (SFA/C18:2¶)				
mean	0.06	0.24	0.32	0.52
SD	0.31	0.33	0.41	0.35
C (C12:0-C16:0/PUFA)**				
mean	-0.01	0.20	0.20	0.36
SD	0.30	0.30	0.36	0.31
D (C12:0-C16:0/C18:2)				
mean	0.02	0.18	0.22	0.35
SD	0.28	0.29	0.37	0.30
E (C12:0-C18:0††/PUFA)				
mean	-0.01	0.26	0.24	0.46
SD	0.34	0.32	0.39	0.36
F (C12:0-C18:0/C18:2)				
mean	0.02	0.24	0.26	0.46
SD	0.31	0.31	0.40	0.33
Measured change				
Plasma total cholesterol				
mean	0.17	0.07	0.30	0.43
SD	0.49	0.47	0.65	0.54

* Keys equation (Keys, Anderson & Grande, 1965b) calculated using different fatty acid combinations (A-F)
[a] Baseline for Red meat diet - Baseline for chicken-fish diet
[b] Red meat diet - Chicken-fish diet
[c] Baseline - Red meat diet
[d] Baseline - Chicken-fish diet
† Total saturated fatty acids
# Total polyunsaturated fatty acids
§ Standard deviation
¶ Linoleic acid
** Lauric, myristic and palmitic acids
†† Lauric, myristic, palmitic and stearic acids

Table 5.11. Difference between predicted change in plasma total cholesterol (mmol/L) level (Keys equation) and measured change in plasma total cholesterol (n = 37)

Keys equation*	Diet			
	Baseline[a]	Treatment[b]	Red meat[c]	Chicken-fish[d]
A (SFA†/PUFA#)				
mean	-0.01	-0.10	0.12	0.25
SD§	0.52	0.50	0.75	0.60
p-value	0.9941	0.3606	0.3766	0.0146
B (SFA/C18:2¶)				
mean	-0.07	-0.17	0.05	0.18
SD	0.55	0.51	0.77	0.60
p-value	0.4905	0.0898	0.7060	0.0759
C (C12:0-C16:0/PUFA)**				
mean	0.03	-0.12	0.10	0.23
SD	0.53	0.51	0.76	0.60
p-value	0.8418	0.2868	0.4719	0.0184
D (C12:0-C16:0/C18:2)				
mean	-0.09	-0.19	0.04	0.16
SD	0.56	0.52	0.77	0.60
p-value	0.3528	0.0660	0.7958	0.0733
E (C12:0-C18:0††/PUFA)				
mean	-0.09	-0.18	0.04	0.17
SD	0.54	0.53	0.77	0.61
p-value	0.3606	0.0898	0.7844	0.1023
F (C12:0-C18:0/C18:2)				
mean	0.07	-0.17	0.06	0.18
SD	0.53	0.52	0.76	0.60
p-value	0.4812	0.1091	0.6950	0.0614

* Keys equation (Keys, Anderson & Grande, 1965b) calculated using different fatty acid combinations (A-F)
[a] Baseline for red meat diet - Baseline for chicken-fish diet
[b] Red meat diet - Chicken-fish diet
[c] Baseline - Red meat diet
[d] Baseline - Chicken-fish diet
† Total saturated fatty acids
# Total polyunsaturated fatty acids
§ Standard deviation
¶ Linoleic acid
** Lauric, myristic and palmitic acids
†† Lauric, myristic, palmitic and stearic acids

5.3 Discussion

With the exception of one subject all the subjects who entered this clinical cross-over study had elevated plasma TC at the onset. They could therefore be classified as having a moderate or high risk for the development of CHD according to the guidelines of the Heart Foundation of Southern Africa (Rossouw *et al.*, 1988). Regression to the mean could clearly be observed since at recruitment only one of the subjects had a normal plasma TC level but with remeasurement during the baseline period of Phase 1 the plasma TC levels of more subjects fell within normal limits. This demonstrates the importance of a baseline period and repeated measurements before any dietary intervention or treatment is introduced.

At baseline the mean plasma TC concentrations of all three study groups were above the level of ≤ 5.20 mmol/L, which is regarded as the desirable level for TC concentrations (Adult Treatment Panel II, 1994). The major impact of dietary intervention on lowering the risk of CHD in terms of plasma TC is clearly demonstrated by this study. Even in those with severely elevated plasma TC a shift to a lower risk in terms of plasma TC could be observed during the treatment periods and plasma TC levels tended to return to higher levels after dietary intervention.

Total cholesterol, LDL-C and apo B lipoprotein are associated with CHD (Adult Treatment Panel II, 1994; Sharrett *et al.*, 1994; Ginsberg, 1990; Sniderman *et al.*, 1982) and in this study TC, LDL_1-C, LDL_2-C, LDL_1-apo B, and LDL_2-apo B were significantly lower during the treatment periods than during the baseline periods, which confirms the beneficial effect of changing from a Western type diet to a prudent diet. This study therefore also confirmed that dietary intervention should be the first step in the treatment of hypercholesterolaemia.

The study further showed that significant changes could be observed within six weeks. The treatment period of six weeks is probably more than adequate to achieve a maximum change in lipoprotein levels with dietary intervention. Morgan, Sinclair & O'Dea (1993) indicated that serum lipoprotein levels seem to stabilize within 10 to 14 days.

Both lipid-lowering diets had a beneficial effect irrespective of whether lean red meat or chicken (without skin) and fish was consumed on the prescribed prudent diet. There were, however,

indications that the CFD was more beneficial than the RMD since the plasma TC of more subjects decreased on the CFD than on the RMD. In the interpretation of the effect of the results it should be kept in mind that, with the exception of sausage, the red meat was very lean (A1). Fat was trimmed and the chicken was without skin. O'Dea *et al.* (1990) showed that it was the beef fat and not the beef itself which has a cholesterol-elevating effect.

Flynn *et al.* (1981 and 1982) could not show a difference in TC, in free-living normolipidaemic subjects, when diets containing beef and pork were compared with diets of poultry and fish. In this study the TC did not differ significantly but HDL-C was higher on the RMD than on the CFD. This is in agreement with the finding of Scott *et al.* (1991). Flynn *et al.* (1982) showed changes in HDL-C both upward and downward with three diets of either beef, or poultry and fish, and pork. The higher HDL-C on the RMD could be explained by the higher intake of total fat and cholesterol on the RMD than on the CFD as well as the lower P/S ratio of the RMD. Total fat (Mensink & Katan, 1987; Ferro-Luzzi *et al.*, 1984) and dietary cholesterol intake (Katan *et al.*, 1986) are positively associated with HDL-C. Very low fat diets (< 10%E) tend to lower LDL-C as well as HDL-C (Morgan, Sinclair & O'Dea, 1993; Sanders *et al.*, 1994) but a moderate fat diet (30%E), low in saturated fat, only seems to lower LDL-C (Morgan, Sinclair & O'Dea, 1993). The difference in HDL-C between the RMD and the CFD was, however, very small and of questionable biological significance. Although diastolic blood pressure was lower on the CFD than on the RMD the difference was very small (1.6 mm Hg) but there are indications in the literature that the intake of n-3 PUFAs lowers blood pressure (Kinsella, Lokesh & Stone, 1990). In this study the intake of n-3 fatty acids was significantly higher on the CFD than on the RMD. Although no other significant direct treatment effects, than HDL-C and blood pressure, were observed between the RMD and the CFD, there are indications that the CFD might have a more favourable effect on plasma lipoproteins than the RMD (Table 5.7). A study on 28 free-living subjects with cholesterol levels < 8 mmol/L showed that mean plasma TC, LDL-C, VLDL-C, VLDL-TAG and plasma TAG concentrations were significantly lower on a diet containing fatty fish as the only "meat" in the diet compared to a diet containing red meat as the only "meat" in the diet (Wolmarans *et al.*, 1991). In the study of Wolmarans *et al.* (1991) no special effort was made to ensure that lean red meat was used and the background diet was a Western type diet.

Results discussed in Chapter 4 indicate that the subjects followed the dietary prescriptions for

the CFD slightly better than for the RMD (Tables 4.6 and 4.7) and this could have concealed significant differences between the effect of the two lipid-lowering diets. However, non-compliance on the RMD was of a mild nature. Some of the subjects found it difficult to consume red meat seven days per week and they violated the dietary prescriptions more often on the RMD than on the CFD. Only 5.3% of the subjects who participated in this study indicated that they would have liked to eat red meat five times per week while the rest preferred to have it less often. South Africans following a Western type diet seem to be less fond of red meat than is generally believed. However, this study was undertaken in an urban community whose dietary preferences might differ from those of rural areas.

Several combinations of SFAs and PUFAs were used in calculating the Keys equation in order to determine which combination predicted the change in plasma TC best. Saturated fatty acids with ten or less carbons atoms in the chain and stearic acid does not have a cholesterol-elevating effect (Tholstrup *et al.*,1994a). The inclusion of the short chain SFAs and stearic acid did not seem to have a negative effect on the ability of the Keys equation to estimate the change in plasma TC in response to a change in dietary SFAs (%E) and PUFAs (%E) and dietary cholesterol in this study. The equation to predict the response of cholesterol concentrations to changes in fatty acids and cholesterol intake apply more to groups than to individuals (Grundy & Vega, 1988). Nevertheless, only when the change in plasma TC between the baseline diet and the CFD was estimated did the predicted and measured change in plasma TC differed significantly. The measured change in plasma TC between the baseline period and the CFD was higher than predicted. This might indicate that the intake of the long chain PUFAs on the CFD influenced the ability of the Keys equation to predict the change in plasma TC as a result of a change in dietary fat intake.

The lack of significant differences in plasma lipids and lipoproteins between the reference diet and the RMD as well as the limited number of significant differences between the reference diet and the CFD are disappointing. For the reference diet the mean intake of the total fat varied between 31.1 and 34.5%E during the study. Although these figures are in general higher than the mean fat intake on the RMD and the CFD (25.8 to 31.0%E), it was lower than the baseline total fat intake (35.4 to 38.2%E) of the treatment groups (Tables 4.9, 4.10 and 4.11). In the Coronary Risk Factor Intervention Study (CORIS) a mean fat intake of approximately 36%E was reported for men and women, 20 - 44 years of age (Wolmarans *et al.*, 1988). Due to

information sessions which had to be held on the implementation of the treatment diets, subjects knew whether they were in the reference group or in the treatment groups before they kept the first dietary record. Since everybody in the reference group knew that they had elevated plasma TC levels it is possible that they might have changed their dietary intake unconsciously throughout the study. This could have masked significant differences between the two treatment groups and the reference group.

Although the drop out rate in this study could be regarded as high (25%), this probably did not have any negative impact on the outcome of the study. The most important requirement for this study on a free-living population was dietary compliance. Those subjects who completed the study could be regarded as "good" compliers since the majority of the subjects who withdrew from the study did so because they did not want to comply with the dietary prescriptions. Results reported in Chapter 4 confirmed that in this study non-compliance of those who followed the treatment diets was of a mild nature.

The cross-over design of the study compensated for the high drop out rate. Treatment effects were estimated within subjects rather than between subjects. Since the variability of measurements taken on different subjects is usually far greater than the variability of repeated measurements taken on the same subject the cross-over design is statistically more powerful and optimal. With the exception of VLDL-TAG which could have been influenced by the non-fasting of subjects no differences in baseline comparisons or in direct-by-period interaction effect were observed which allowed for using the second treatment period value for analysis of the direct treatment effect.

5.4 Conclusions

* In conclusion, this study showed that it is possible to change the Western type diet of free-living subjects to a prudent diet, and that the prudent diets, one containing lean red meat and the other containing chicken (without skin) and fish, lowered plasma TC within six weeks. There were indications that the CFD might be slightly more beneficial in the treatment of elevated plasma TC than the RMD because more subjects showed a lowering of plasma TC levels on the CFD than on the RMD.

* Calculations based on the Keys equation showed that TC will not differ between a prudent diet containing lean red meat and a prudent diet containing chicken (without skin) and fish. This was confirmed by analysis of the data within the cross-over design of the study. With the exception of HDL-C, which was significantly higher on the RMD than on the CFD, no other significant differences were observed in the direct treatment effect of the two diets. The higher intake of total fat and dietary cholesterol, as well as the lower P/S ratio on the RMD than on the CFD, could explain the higher ($p < 0.05$) HDL-C on the former diet. Some of the atherogenic lipoproteins were lower on the CFD than on the RMD, suggesting a slightly more favourable effect of the CFD on lipoproteins. The differences, however, were not significant and probably not of biological significance.

* Therefore, in agreement with Watts *et al.* (1988), this study showed that, depending on energy needs (7 500 to 14 000 kJ), a daily portion of lean red meat varying between 120 g and 210 g, could be included in a lipid-lowering diet. This recommendation is in line with the recommendations in the New Zealand dietary guidelines as well as the recommendations in the NCEP (Mann *et al.*, 1993; Adult Treatment Panel II, 1994).

* Although it has not been addressed in detail in the *Discussion* no differences in direct-by-period interaction effect was observed. It was therefore possible to analyse the data for the direct treatment effect.

CHAPTER 6

THE EFFECT OF DIET ON THE FATTY ACID COMPOSITION OF PLASMA TRIACYLGLYCEROL AND CHOLESTERYL ESTER

6.1 Introduction

The type of fat in the diet influences the fatty acid composition of the plasma (Vessby *et al.*, 1980b; Moilanen *et al.*, 1983), and the different plasma lipid classes are characterised by a distinct fatty acid composition (Lindgren, Nichols & Wills, 1961). The fatty acid that predominates in the TAG is oleic acid (C18:1n-9) while linoleic acid (C18:2n-6) predominates in the CE (Lindgren, Nichols & Wills, 1961). In addition to these fatty acids, palmitic (C16:0) and linoleic acids in TAG, and oleic, palmitic and arachidonic acids (C20:4n-6, AA) in CE are also present in appreciable amounts (Lindgren, Nichols & Wills, 1961).

While the fatty acid composition of TAG reflects more recent intakes of the type of fat in the diet, the fatty acid composition of CE is an indication of medium-term (Vessby *et al.*, 1980b) and adipose tissue of long-term (2-3 years) intake of dietary fat composition (Beynen, Hermus & Hautfast, 1980; Van Staveren *et al.*, 1986). In patients with hyperlipoproteinaemia an increase in the P/S ratio from 0.2 to 2.0 resulted in a change in fatty acid composition of TAG within one day while the change in fatty acid composition of the CE was more continuous over ten days (Vessby *et al.*, 1980a).

Linoleic acid and α-linolenic acid (C18:3n-3), the essential fatty acids, are not synthesised in the body but must be provided by the diet (Mahan & Arlin, 1992) and their levels in the fatty acids in serum are therefore an indication of PUFA intake. An increase in the P/S ratio of the diet resulted in an increase in the linoleic acid content of CE and TAG (Vessby *et al.*, 1980b; Moilanen *et al.*, 1983; Tremoli *et al.*, 1986), and a positive correlation ($r=0.70$) between dietary linoleic acid intake (g/day) and the percentage of linoleic acid in plasma CE has been shown (James *et al.*, 1993). A good correlation between the percentage of linoleic acid in CE and TAG was found by Nikkari *et al.* (1983b). Studies have also shown that an increase in the

intake of the n-3 PUFA, EPA and DHA (C22:6n-3), was reflected in the CE and TAG of serum and plasma (Boberg, Vessby & Selinus, 1986; Leaf *et al.*, 1995).

The importance of the n-6/n-3 (linoleic/α-linolenic) ratio of the diet is receiving much attention (Simopoulus, 1989; Horrobin, 1991). The Western type diet is characterised by an n-6/n-3 ratio of 20:1 (Shrapnel *et al.*, 1994) while it is suggested that this ratio of linoleic acid to α-linolenic acid should be between 5:1 and 10:1 (Galli, 1995). According to Horrobin (1991) the n-3 PUFAs, EPA and DHA, and the n-6 PUFAs, gamma-linolenic acid (C18:3n-6, GLA), dihomo-gamma-linolenic acid (C20:3n-6, DGLA) and arachidonic acid (AA), which are all past the rate-limiting 6-desaturation step and at equivalent stages of desaturation, should be compared. In contrast, C18:2n-6 and C18:3n-3 should be compared. The intake of the long chain n-6 PUFAs on the Western type diet is estimated to be between 200-600 mg while the intake of the long chain n-3 PUFAs is approximately 50-86 mg (Horrobin, 1991) making the discrepancy in the ratio not so large. The consumption of n-3 rich foods, such as green leafy vegetables, legumes and fish, is recommended for those with a linoleic to α-linolenic ratio greater than 10:1 (Galli, 1995).

Saturated fatty acids are also synthesized in the body (Leaf, 1995; Lands, 1995) and a high intake of carbohydrate can lead to the synthesis of palmitic acid and palmitoleic acid (Sanders *et al.*, 1994; Lands, 1995). The SFA composition of the plasma may therefore not be a good reflection of dietary intake of SFAs (Simon *et al.*, 1995). This probably also applies to MUFAs (Sanders *et al.*, 1994; Emken, 1992; Lands, 1995). Oleic acid, a MUFA, was found to be significantly higher in the plasma CE of normocholesterolaemic subjects on a low fat (<10%E) diet compared to a high fat (33%E) diet. This might indicate that oleic acid is synthesized from carbohydrate. In addition, the SFA, stearic acid (C18:0), can be desaturated in the body to form oleic acid (Emken, 1992).

A negative association between serum TC and the percentage of linoleic acid in CE (-0.242) was found in eight-year-old Finnish boys (Nikkari *et al.*, 1983a). Moilanen *et al.* (1986) also found that the percentage of linoleic acid in the CE correlated inversely with total and LDL-C while most SFAs, MUFAs and n-3 fatty acids correlated positively with total and LDL-C in Finnish boys who were 3 to 18 years of age. HDL-C correlated positively with linoleic acid in the CE and negatively with palmitic acid in CE (Moilanen *et al.*, 1986). A linear increase in the

HDL-C to TC ratio was found with an increase in the percentage of linoleic acid in CE (Moilanen *et al.*, 1986). The relative proportions of linoleic acid and arachidonic acid in all lipid fractions of hyperlipidaemic subjects were found to be smaller than in subjects with normal lipids (Schrade, Biegler & Böhle, 1961). SFAs were positively associated with CHD in a case-control study on men who participated in the MRFIT study (Simon *et al.*, 1995). The stearic acid content of the serum CE of the subjects in this trial was significantly higher in CHD cases than in controls (Simon *et al.*, 1995). Palmitic acid in the CE was also positively associated with CHD even after controlling for its effect on plasma lipids.

The above indicates that a qualitative change in dietary fat intake influences the fatty acid composition of plasma TAG and CE and could play a role in the development of CHD. A change in the P/S ratio of the diet could be expected when beef and mutton are replaced by chicken and fish since the P/S ratio of the fat in the latter is higher than that of red meat (Gouws & Langenhoven, 1986a).

The aim of this chapter therefore is to report on the effect of two prudent diets, which differed only in the type of "meat" (lean red meat versus chicken, without skin, and fish) on the fatty acid composition of plasma TAG and CE of free-living subjects with age related elevated plasma cholesterol levels.

The methods used for the analysis of the fatty acid composition of the TAG and CE are discussed in detail in Chapter 3.

6.2 Results

6.2.1 Fatty acid composition of plasma triacylglycerols and cholesteryl ester

6.2.1.1 Mean values

An analysis of the fatty acid composition of the plasma TAGs and CEs are given in Tables 6.1 to 6.6 for Study Groups 1, 2 and 3. The mean percentage of oleic acid was lower in the RMD and CFD treatment periods than in the baseline periods (Tables 6.1, 6.2, 6.4 and 6.5). In the reference group, however, the oleic acid composition of the TAG and CE showed very little change during the study (Tables 6.3 and 6.6). The percentage of linoleic acid in the TAG

Table 6.1. Mean (and standard deviation) of fatty acid composition of plasma triacylglycerols (TAG) of the subjects in the baseline and treatment periods (Study Group 1, n = 21)

		Diets			
		Red meat		Chicken-fish	
Fatty acid (%)		B_1*	T_1†	B_2#	T_2§
TAG C16:0	mean	23.15	23.00	24.16	22.55
	SD¶	3.86	2.80	3.47	3.85
TAG C16:1	mean	2.90	3.47	3.66	3.84
	SD	0.84	1.40	1.31	1.04
TAG C18:0	mean	4.38	3.27	3.51	3.01
	SD	1.35	0.67	1.01	0.64
TAG C18:1	mean	39.83	38.96	39.08	36.42
	SD	4.20	2.83	3.11	3.96
TAG C18:2	mean	24.54	27.26	25.63	29.19
	SD	4.74	4.98	4.58	5.79
TAG C18:3	mean	0.44	0.36	0.45	0.42
	SD	0.11	0.09	0.12	0.16
TAG C20:3	mean	0.32	0.32	0.35	0.37
	SD	0.13	0.10	0.10	0.08
TAG C20:4	mean	2.32	1.80	1.50	1.53
	SD	1.12	0.51	0.49	0.47
TAG C20:5	mean	0.42	0.19	0.20	0.38
	SD	0.46	0.13	0.10	0.26
TAG C22:5	mean	0.35	0.33	0.39	0.50
	SD	0.17	0.15	0.18	0.23
TAG C22:6	mean	1.35	1.03	1.07	1.77
	SD	0.42	0.38	0.41	0.92
TAG EPA/AA**	mean	0.16	0.11	0.14	0.26
	SD	0.15	0.06	0.06	0.21
TAG P/S††	mean	1.11	1.21	1.08	1.38
	SD	0.28	0.33	0.25	0.51

* Baseline period Phase 1
† Treatment period Phase 1
\# Baseline period Phase 2
§ Treatment period Phase 2
¶ Standard deviation
** Eicosapentaenoic acid to arachidonic acid ratio
†† Polyunsaturated fatty acid to saturated fatty acid ratio

Table 6.2. Mean (and standard deviation) of fatty acid composition of plasma triacylglycerols (TAG) of the subjects in the baseline and treatment periods (Study Group 2, n = 18)

		Diets			
		Chicken-fish		Red meat	
Fatty acid (%)		B_1*	T_1†	B_2#	T_2§
TAG C16:0	mean	23.88	21.97	23.05	22.99
	SD¶	2.85	2.15	3.08	2.96
TAG C16:1	mean	3.62	3.45	3.28	4.04
	SD	1.45	1.27	1.18	1.05
TAG C18:0	mean	4.01	2.97	3.63	3.60
	SD	1.09	0.85	1.24	1.11
TAG C18:1	mean	40.53	37.10	40.45	39.35
	SD	4.21	3.77	4.68	3.60
TAG C18:2	mean	23.74	29.97	25.68	26.57
	SD	5.20	4.69	6.89	5.69
TAG C18:3	mean	0.35	0.35	0.52	0.41
	SD	0.21	0.13	0.25	0.08
TAG C20:3	mean	0.36	0.28	0.35	0.36
	SD	0.19	0.12	0.12	0.15
TAG C20:4	mean	1.77	1.80	1.42	1.41
	SD	0.37	0.40	0.41	0.49
TAG C20:5	mean	0.20	0.29	0.21	0.16
	SD	0.21	0.12	0.11	0.10
TAG C22:5	mean	0.26	0.32	0.38	0.26
	SD	0.18	0.15	0.24	0.16
TAG C22:6	mean	1.27	1.49	1.03	0.85
	SD	0.37	0.39	0.52	0.46
TAG EPA/AA**	mean	0.13	0.16	0.15	0.11
	SD	0.16	0.06	0.06	0.06
TAG P/S ratio††	mean	1.02	1.38	1.15	1.16
	SD	0.27	0.26	0.47	0.36

* Baseline period Phase 1
† Treatment period Phase 1
\# Baseline period Phase 2
§ Treatment period Phase 2
¶ Standard deviation
** Eicosapentaenoic acid to arachidonic acid ratio
†† Polyunsaturated fatty acid to saturated fatty acid ratio

Table 6.3. Mean (and standard deviation) of fatty acid composition of plasma triacylglycerols (TAG) of the subjects in the baseline and treatment periods (Study Group 3, n = 13)

		Diets			
		Habitual		Habitual	
Fatty acid (%)		B_1*	T_1†	B_2#	T_2§
TAG C16:0	mean	23.93	24.69	25.34	25.10
	SD¶	2.14	2.78	2.74	2.55
TAG C16:1	mean	4.18	3.94	4.15	4.45
	SD	1.59	1.64	1.29	0.98
TAG C18:0	mean	3.74	3.63	3.75	3.45
	SD	0.65	0.96	1.16	0.93
TAG C18:1	mean	40.91	39.97	40.53	40.52
	SD	2.57	2.14	1.87	2.54
TAG C18:2	mean	22.40	22.29	22.17	22.47
	SD	4.10	4.83	4.84	4.71
TAG C18:3	mean	0.51	0.43	0.48	0.41
	SD	0.15	0.23	0.15	0.11
TAG C20:3	mean	0.37	0.28	0.31	0.28
	SD	0.13	0.07	0.09	0.12
TAG C20:4	mean	1.68	1.82	1.48	1.31
	SD	0.48	0.52	0.50	0.37
TAG C20:5	mean	0.37	0.43	0.23	0.26
	SD	0.42	0.57	0.12	0.19
TAG C22:5	mean	0.36	0.40	0.41	0.36
	SD	0.14	0.28	0.17	0.17
TAG C22:6	mean	1.54	2.14	1.14	1.40
	SD	0.42	2.18	0.53	0.69
TAG EPA/AA**	mean	0.30	0.22	0.16	0.21
	SD	0.51	0.25	0.08	0.16
TAG P/S††	mean	0.99	0.99	0.91	0.94
	SD	0.22	0.24	0.25	0.26

* Baseline period Phase 1
† Treatment period Phase 1
\# Baseline period Phase 2
§ Treatment period Phase 2
¶ Standard deviation
** Eicosapentaenoic acid to arachidonic acid ratio
†† Polyunsaturated fatty acid to saturated fatty acid ratio

Table 6.4. Mean (and standard deviation) of fatty acid composition of plasma cholesteryl esters (CE) of the subjects in the baseline and treatment periods (Study Group 1, n = 21)

		Diets			
		Red meat		Chicken-fish	
Fatty acid (%)		B_1*	T_1†	B_2#	T_2§
CE C16:0	mean	11.05	11.67	11.24	11.66
	SD¶	1.83	1.29	0.78	1.40
CE C16:1	mean	1.88	2.56	2.13	2.20
	SD	0.44	0.99	0.93	0.90
CE C18:0	mean	1.25	0.97	1.15	0.90
	SD	0.29	0.19	0.25	0.19
CE C18:1	mean	20.77	19.35	19.30	17.97
	SD	2.21	1.95	1.76	2.06
CE C18:2	mean	57.79	58.26	59.40	60.64
	SD	2.97	3.47	3.30	4.27
CE C18:3	mean	0.19	0.10	0.26	0.15
	SD	0.09	0.10	0.12	0.06
CE C20:3	mean	0.46	0.44	0.46	0.42
	SD	0.18	0.14	0.13	0.12
CE C20:4	mean	5.62	5.91	5.23	5.14
	SD	1.41	1.45	1.06	1.15
CE C20:5	mean	0.52	0.32	0.35	0.43
	SD	0.28	0.15	0.19	0.22
CE C22:5	mean	0	0	0.001	0.002
	SD	0	0	0.007	0.009
CE C22:6	mean	0.47	0.42	0.47	0.48
	SD	0.16	0.15	0.19	0.19
CE EPA/AA**	mean	0.10	0.05	0.07	0.09
	SD	0.06	0.02	0.03	0.04
CE P/S††	mean	5.45	5.25	5.37	5.46
	SD	1.06	0.73	0.52	0.93

* Baseline period Phase 1
† Treatment period Phase 1
\# Baseline period Phase 2
§ Treatment period Phase 2
¶ Standard deviation
** Eicosapentaenoic acid to arachidonic acid ratio
†† Polyunsaturated fatty acid to saturated fatty acid ratio

Table 6.5. Mean (and standard deviation) of fatty acid composition of plasma cholesteryl esters (CE) of the subjects in the baseline and treatment periods (Study Group 2, n = 18)

		Diets			
		Chicken-fish		Red meat	
Fatty acid (%)		B_1*	T_1†	B_2#	T_2§
CE C16:0	mean	11.03	11.98	11.53	11.83
	SD¶	1.90	1.09	1.19	1.51
CE C16:1	mean	1.86	2.00	2.03	2.10
	SD	0.60	0.67	1.01	0.86
CE C18:0	mean	1.35	0.92	1.25	1.13
	SD	0.42	0.27	0.31	0.25
CE C18:1	mean	20.07	17.93	19.76	18.77
	SD	3.11	2.17	2.37	2.22
CE C18:2	mean	58.43	60.37	58.72	59.58
	SD	4.74	3.15	3.95	4.42
CE C18:3	mean	0.20	0.11	0.28	0.17
	SD	0.08	0.08	0.13	0.05
CE C20:3	mean	0.56	0.48	0.48	0.49
	SD	0.14	0.18	0.14	0.14
CE C20:4	mean	5.55	5.15	5.11	5.30
	SD	1.08	1.04	1.21	0.95
CE C20:5	mean	0.44	0.53	0.39	0.26
	SD	0.28	0.25	0.28	0.11
CE C22:5	mean	0	0	0	0
	SD	0	0	0	0
CE C22:6	mean	0.50	0.55	0.45	0.37
	SD	0.16	0.14	0.13	0.09
CE EPA/AA**	mean	0.08	0.10	0.08	0.05
	SD	0.05	0.05	0.06	0.02
CE P/S††	mean	5.50	5.25	5.19	5.21
	SD	1.25	0.59	0.74	0.94

* Baseline period Phase 1
† Treatment period Phase 1
\# Baseline period Phase 2
§ Treatment period Phase 2
¶ Standard deviation
** Eicosapentaenoic acid to arachidonic acid ratio
†† Polyunsaturated fatty acid to saturated fatty acid ratio

Table 6.6. Mean (and standard deviation) of fatty acid composition of plasma cholesteryl esters (CE) of the subjects in the baseline and treatment periods (Study Group 3, n = 13)

		Diets			
		Habitual		Habitual	
Fatty acids (%)		B_1*	T_1†	B_2#	T_2§
CE C16:0	mean	11.61	11.58	12.47	12.12
	SD¶	1.22	1.02	1.19	1.30
CE C16:1	mean	3.06	3.47	2.96	3.31
	SD	1.10	1.42	1.19	1.56
CE C18:0	mean	1.26	1.03	1.12	1.12
	SD	0.24	0.37	0.26	0.30
CE C18:1	mean	21.18	21.29	21.42	21.21
	SD	2.75	2.44	2.32	2.07
CE C18:2	mean	55.30	55.11	55.27	54.67
	SD	4.35	3.74	4.19	4.34
CE C18:3	mean	0.19	0.13	0.32	0.21
	SD	0.14	0.13	0.24	0.07
CE C20:3	mean	0.53	0.48	0.51	0.54
	SD	0.17	0.19	0.13	0.19
CE C20:4	mean	5.86	5.90	5.14	5.77
	SD	1.22	1.47	1.35	1.08
CE C20:5	mean	0.56	0.51	0.41	0.59
	SD	0.28	0.28	0.21	0.45
CE C22:5	mean	0	0	0	0.004
	SD	0	0	0	0.01
CE C22:6	mean	0.46	0.49	0.39	0.46
	SD	0.20	0.15	0.13	0.19
CE EPA/AA**	mean	0.09	0.09	0.08	0.10
	SD	0.03	0.04	0.05	0.06
CE P/S††	mean	4.95	5.01	4.62	4.77
	SD	0.70	0.65	0.68	0.72

* Baseline period Phase 1
† Treatment period Phase 1
\# Baseline period Phase 2
§ Treatment period Phase 2
¶ Standard deviation
** Eicosapentaenoic acid to arachidonic acid ratio
†† Polyunsaturated fatty acid to saturated fatty acid ratio

showed a tendency to increase on both the red meat and the chicken-fish treatment diets (Tables 6.1 and 6.2). This finding was also true for the percentage of linoleic acid in the CE (Tables 6.4 and 6.5). Results, however, showed that the percentage of linoleic acid in the TAG and the plasma CE stayed more or less constant in the reference group (Tables 6.3 and 6.6). The EPA content (%) of the TAG as well as the CE showed that the percentage of EPA in TAG tended to be lower on the RMD and higher on the CFD than in the baseline periods. The difference between the baseline period and the CFD, however, seemed to be smaller than the difference between the baseline period and the RMD for Study Group 1. Differences in the percentage of EPA in the baseline period and the treatment periods of the two treatment groups were more pronounced in the CE (Tables 6.4 and 6.5) than in the TAG.

6.2.1.2 Baseline versus treatment periods

A negative result for the difference in the plasma fatty acid composition in the baseline period and treatment period indicates that the value was higher in the baseline period than in the treatment period (Tables 6.7 and 6.8). The percentage of stearic acid and oleic acid in the plasma TAG and the CE tended to be lower on the treatment diets than on the baseline diet. On the RMD and CFD the percentage of linoleic acid in the TAG was significantly higher than on the baseline diet. In the CE there was, however, no significant difference in the percentage of linoleic acid between the baseline and treatment diets.

6.2.1.3 Cross-over analysis

In Tables 6.9 and 6.10 the p-values for the differences between the baseline comparisons (carry-over effect) and the direct-by-period interaction effect are given. Significant carry-over effects (baseline comparisons) for C16:1 and C18:3 in the plasma TAGs were observed (Table 6.9). No other significant differences for the baseline comparisons or for the direct-by-period interaction effect were observed for the fatty acid composition of the plasma TAGs. No significant first order carry-over effects (baseline comparisons) or second carry-over effects (direct-by-period interaction effects) were observed for the fatty acid composition of the cholesteryl esters (Table 6.10).

6.2.1.4 Direct treatment effect

Significant differences between the RMD and the CFD were observed for the fatty acid composition of the plasma TAG and the CE (Tables 6.11 and 6.12). Stearic acid and oleic acid

intake was significantly higher on the RMD than on the CFD while linoleic acid, EPA, C22:5n-3 and DHA were significantly higher on the CFD than on the RMD in the TAG. A similar picture was observed for the CE, but the percentage of palmitoleic acid as well as arachidonic acid was also higher on the RMD than on the CFD and no difference between the two treatment diets was found for C22:5n-3. In the plasma TAG the P/S ratio and the EPA/arachidonic acid (EPA/AA) ratio, but in the CE only the EPA/AA ratio, were significantly higher on the CFD than on the RMD.

6.2.1.5 Treatment diet versus reference diet

Estimated differences between the changes in the RMD and the reference diet and between the CFD and the reference diet are given in Tables 6.13 and 6.14 for the plasma TAG, and in Tables 6.15 and 6.16 for the plasma CE. A negative result indicates an increase over time in the reference group and a decrease over time in the treatment group. This results in a higher value on the reference diet than on the treatment diet. The opposite is true of a positive result. The significant differences observed for the RMD versus the reference diet were a negative result for DHA in the TAG, and for EPA, DHA and the EPA/AA ratio in the CE (Tables 6.13 and 6.15). The difference between the CFD and the reference diet showed a negative result for palmitic acid and positive results for linoleic acid and the P/S ratio in the TAG fraction (Table 6.14). No significant differences were observed in the CE for the difference between the CFD and the reference diet (Table 6.16).

6.2.2 Correlation between dietary intake of fatty acids and the percentage thereof in plasma triacylglycerol and cholesteryl ester

Positive correlations between the dietary intake of linoleic acid and the percentage of linoleic acid in the plasma TAG and CE were found, especially in Study Group 1 on the RMD in Phase 1, and in the TAG in Study Group 1 on the CFD in Phase 2 (Table 6.17). EPA intake correlated positively with the percentage of EPA in the CE in Study Group 2 on the CFD in Phase 1. No correlation between EPA intake and the percentage thereof in the plasma TAG or CE was found in Study Group 1 on the CFD in Phase 2. A negative correlation between linoleic acid intake and the percentage of EPA in the plasma CE was found in Phase 2 with the RMD while these parameters correlated positively in Phase 2 with the CFD.

Table 6.7. Difference in fatty acid composition of plasma triacylglycerols (TAG) of subjects between the baseline and treatment periods (treatment period value - baseline value)

		Diets			
		Red meat		Chicken-fish	
Fatty acids (%)		n = 37	p-value	n = 37	p-value
TAG C16:0	mean	-0.79	0.0869	-0.88	0.0020
	SD*	2.89		3.10	
TAG C16:1	mean	0.19	0.3726	0.40	0.1108
	SD	1.34		1.31	
TAG C18:0	mean	-1.06	0.0001	-0.29	0.0991
	SD	1.18		1.22	
TAG C18:1	mean	-2.03	0.0036	-2.10	0.0146
	SD	3.83		5.30	
TAG C18:2	mean	4.27	0.0001	2.63	0.0066
	SD	5.86		7.49	
TAG C18:3	mean	-0.04	0.1089	-0.07	0.0470
	SD	-0.18		0.22	
TAG C20:3	mean	-0.04	0.1828	0.02	0.3337
	SD	0.20		0.12	
TAG C20:4	mean	-0.31	0.0592	0.01	0.9470
	SD	0.97		0.47	
TAG C20:5	mean	-0.10	0.5094	0.04	0.7699
	SD	0.42		0.21	
TAG C22:5	mean	0.01	0.8936	-0.01	0.7225
	SD	0.16		0.25	
TAG C22:6	mean	-0.09	0.4358	0.25	0.1007
	SD	0.55		0.90	

* Standard deviation

Table 6.8. Difference in fatty acid composition of plasma cholesteryl esters (CE) of subjects between the baseline and treatment periods (treatment period value - baseline period value)

		Diets			
		Red meat		Chicken-fish	
Fatty acids (%)		n = 37	p-value	n = 37	p-value
CE C16:0	mean	0.71	0.0614	0.35	0.2294
	SD*	2.28		1.69	
CE C16:1	mean	0.39	0.0096	0.06	0.6220
	SD	0.86		0.95	
CE C18:0	mean	-0.36	0.0001	-0.17	0.0002
	SD	0.41		0.26	
CE C18:1	mean	-1.94	0.0005	-1.19	0.0026
	SD	3.14		2.17	
CE C18:2	mean	1.48	0.0840	1.01	0.1313
	SD	4.52		4.17	
CE C18:3	mean	-0.09	0.0001	-0.09	0.0001
	SD	0.11		0.11	
CE C20:3	mean	-0.04	0.3665	-0.01	0.8412
	SD	0.23		0.15	
CE C20:4	mean	-0.06	0.3848	0.08	0.5435
	SD	1.32		0.92	
CE C20:5	mean	-0.08	0.2206	-0.02	0.8349
	SD	0.34		0.26	
CE C22:5	mean	0.00	-	0.00	1.0000
	SD	0.00		0.00	
CE C22:6	mean	-0.00	0.9877	-0.02	0.4067
	SD	0.22		0.17	

* Standard deviation

Table 6.9. P-values for the differences between the baseline comparisons (carry-over effect) and the direct-by-period interaction effect for Study Groups 1 and 2: fatty acid composition of plasma triacylglycerol (TAG) of subjects

Fatty acid (%)	Baseline comparisons (Carry-over effect)	Direct-by-period interaction effect
	p-value	p-value
TAG C16:0	0.0731	0.8834
TAG C16:1	0.0159	0.7815
TAG C18:0	0.2317	0.3548
TAG C18:1	0.6203	0.6541
TAG C18:2	0.6398	0.8012
TAG C18:3	0.0127	0.9680
TAG C20:3	0.2970	0.3130
TAG C20:4	0.1364	0.0693
TAG C20:5	0.0939	0.6063
TAG C22:5	0.2334	0.1314
TAG C22:6	0.8271	0.1949
TAG EPA/AA*	0.4140	0.3139
TAG P/S†	0.1623	0.9093

* Eicosapentaenoic acid to arachidonic acid ratio
† Polyunsaturated fatty acid to saturated fatty acid ratio

Table 6.10. P-values for the differences between the baseline comparisons (carry-over effect) and the direct-by-period interaction effect for Study Groups 1 and 2: fatty acid composition of plasma cholesteryl esters (CE) of subjects

Fatty acid (%)	Baseline comparisons (carry-over effect)	Direct-by-treatment interaction effect
	p-value	p-value
CE C16:0	0.6257	0.8101
CE C16:1	0.8017	0.2217
CE C18:0	0.9986	0.9119
CE C18:1	0.1659	0.7874
CE C18:2	0.3525	0.6250
CE C18:3	0.8795	0.9247
CE C20:3	0.1996	0.8711
CE C20:4	0.8912	0.4541
CE C20:5	0.3434	0.5231
CE C22:5	0.3615	0.8577
CE C22:6	0.5052	0.9979
CE EPA/AA*	0.1957	0.3417
CE P/S†	0.5424	0.8500

* Eicosapentaenoic acid to arachidonic acid ratio
† Polyunsaturated fatty acid to saturated fatty acid ratio

Table 6.11. Direct treatment effect between red meat and chicken-fish diet: fatty acid composition of plasma triacylglycerol (TAG) of subjects (n =39)*

Fatty acid (%)	Lower confidence limit	Difference between means†	Upper confidence limit	p-value
TAG C16:0	-0.8414	-0.3645	0.1125	0.1301
TAG C16:1	-0.2765	-0.0546	0.1673	0.6210
TAG C18:0	-0.3653	-0.2255	-0.0856	0.0023
TAG C18:1	-1.8619	-1.1963	-0.5308	0.0008
TAG C18:2	0.4771	1.3332	2.1892	0.0032
TAG C18:3	-0.0292	-0.0005	0.0281	0.9700
TAG C20:3	-0.0316	-0.0056	0.0204	0.6632
TAG C20:4	-0.0475	0.0300	0.1075	0.4379
TAG C20:5	0.0450	0.0802	0.1155	0.0001
TAG C22:5	0.0354	0.0594	0.0833	0.0001
TAG C22:6	0.2198	0.3457	0.4716	0.0001
TAG EPA/AA#	0.0251	0.0515	0.0779	0.0003
TAG P/S§	0.0365	0.0986	0.1607	0.0027

* Study Group 1 = Red meat Phase 1; Chicken-fish Phase 2;
Study Group 2 = Chicken-fish Phase 1; Red meat Phase 2.

† Estimated treatment effect; Study Group 1 - Study Group 2; Negative result indicates that the value on the red meat diet is higher than on the chicken-fish diet.

Eicosapentaenoic acid to arachidonic acid ratio

§ Polyunsaturated fatty acid to saturated fatty acid ratio

Table 6.12. Direct treatment effect between red meat and chicken-fish diet: fatty acid composition of cholesteryl esters (CE) of subjects (n = 39)*

Fatty acid (%)	Lower confidence limit	Difference between means†	Upper confidence limit	p-value
CE C16:0	-0.2442	0.0361	0.3163	0.7958
CE C16:1	-0.2200	-0.1165	-0.0131	0.0283
CE C18:0	-0.1192	-0.0712	-0.0233	0.0047
CE C18:1	-0.9046	-0.5544	-0.2042	0.0028
CE C18:2	0.2500	0.7923	1.3345	0.0053
CE C18:3	-0.0196	-0.0030	0.0135	0.7142
CE C20:3	-0.0347	-0.0061	0.0226	0.6714
CE C20:4	-0.4001	-0.2302	-0.0603	0.0093
CE C20:5	0.0524	0.0953	0.1382	0.0001
CE C22:5	-0.0006	0.0005	0.0015	0.3615
CE C22:6	0.0268	0.0579	0.0890	0.0006
CE EPA/AA#	0.0136	0.0216	0.0295	0.0001
CE P/S§	-0.0997	0.0623	0.2244	0.4408

* Study Group 1 = Red meat Phase 1; Chicken-fish Phase 2;
Study Group 2 = Chicken-fish Phase 1; Red meat Phase 2.

† Estimated treatment effect; Study Group 1 - Study Group 2; Negative result indicates that the value on the red meat diet is higher than on the chicken-fish diet.

Eicosapentaenoic acid to arachidonic acid ratio

§ Polyunsaturated fatty acid to saturated fatty acid ratio

Table 6.13. Estimated difference between the changes in the red meat diet and the reference diet: fatty acid composition of the plasma triacylglycerols (TAG) of subjects

Fatty acid (%)	Lower confidence limit	Difference between means*	Upper confidence limit	p-value
TAG C16:0	-2.4192	-0.3622	1.6948	0.7251
TAG C16:1	-0.1614	0.6322	1.4258	0.1159
TAG C18:0	-1.2076	-0.4077	0.3922	0.3109
TAG C18:1	-3.0267	-0.5033	2.0200	0.6904
TAG C18:2	-2.2600	1.7860	5.8330	0.3795
TAG C18:3	-0.1306	-0.0196	0.0914	0.7241
TAG C20:3	-0.0184	0.0695	0.1574	0.1185
TAG C20:4	-0.8134	-0.2685	0.2765	0.3272
TAG C20:5	-0.4243	-0.1868	0.0507	0.1204
TAG C22:5	-0.2095	-0.0633	0.0828	0.3883
TAG C22:6	-1.1239	-0.6795	-0.2351	0.0034
TAG EPA/AA†	-0.1531	-0.0328	0.0876	0.5869
TAG P/S#	-0.2213	0.0482	0.3177	0.7208

* A negative result indicates an increase or decrease over time in the reference group and a decrease in the treatment group.

* A positive result indicates an increase or decrease over time in the reference group and an increase in the treatment group.

† Eicosapentaenoic acid to arachidonic acid ratio

Polyunsaturated fatty acid to saturated fatty acid ratio

Table 6.14. Estimated difference between the changes in the chicken-fish diet and the reference diet: fatty acid composition of the plasma triacylglycerols (TAG) of subjects

Fatty acid (%)	Lower confidence limit	Difference between means*	Upper confidence limit	p-value
TAG C16:0	-3.3983	-1.9983	-0.5983	0.0060
TAG C16:1	-0.7612	-0.0117	0.7378	0.9752
TAG C18:0	-1.2316	-0.5515	0.1285	0.1096
TAG C18:1	-5.1024	-2.5328	0.0367	0.0532
TAG C18:2	1.1490	4.7010	8.2530	0.0105
TAG C18:3	-0.0481	0.0612	0.1705	0.2664
TAG C20:3	-0.0663	0.0377	0.1417	0.4701
TAG C20:4	-0.2270	0.0477	0.3224	0.7288
TAG C20:5	-0.0990	0.0935	0.2859	0.3341
TAG C22:5	-0.0050	0.0959	0.1968	0.0620
TAG C22:6	-0.4925	0,0556	0.6037	0.8393
TAG EPA/AA†	-0.0481	0.0960	0.2400	0.1870
TAG P/S#	0.0880	0.3154	0.5427	0.0075

* A negative result indicates an increase or decrease over time in the reference group and a decrease in the treatment group.

* A positive result indicates an increase or decrease over time in the reference group and an increase in the treatment group.

† Eicosapentaenoic acid to arachidonic acid ratio

Polyunsaturated fatty acid to saturated fatty acid ratio

Table 6.15. Estimated difference between the changes in the red meat diet and the reference diet: fatty acid composition of the plasma cholesteryl esters (CE) of subjects

Fatty acid (%)	Lower confidence limit	Difference between means*	Upper confidence limit	p-value
CE C16:0	-0.5573	0.6694	1.8960	0.2783
CE C16:1	-0.5469	0.0153	0.5774	0.9567
CE C18:0	-0.2567	-0.0926	0.0715	0.2626
CE C18:1	-2.5831	-1.1688	0.2454	0.1032
CE C18:2	-1.2023	1.0385	3.2793	0.3564
CE C18:3	-0.0955	-0.0139	0.0678	0.7348
CE C20:3	-0.1305	0.0022	0.1348	0.9738
CE C20:4	-0.8149	-0.0924	0.6301	0.7983
CE C20:5	-0.4155	-0.2371	-0.0586	0.0103
CE C22:5	-0.0041	-0.0019	0.0003	0.0832
CE C22:6	-0.2237	-0.1173	-0.0109	0.0313
CE EPA/AA†	-0.0714	-0.0404	-0.0095	0.0116
CE P/S#	-0.9108	-0.2051	0.5006	0.5620

* A negative result indicates an increase or decrease over time in the reference group and a decrease in the treatment group.

* A positive result indicates an increase or decrease over time in the reference group and an increase in the treatment group.

† Eicosapentaenoic acid to arachidonic acid ratio

Polyunsaturated fatty acid to saturated fatty acid ratio

Table 6.16. Estimated difference between the changes in the chicken-fish diet and the reference diet: fatty acid composition of the plasma cholesteryl esters (CE) of subjects

Fatty acid (%)	Lower confidence limit	Difference between means*	Upper confidence limit	p-value
CE C16:0	-0.3207	0.8601	2.0410	0.1497
CE C16:1	-0.8103	-0.2806	0.2491	0.2924
CE C18:0	-0.4561	-0.2215	0.0130	0.0636
CE C18:1	-3.3399	-1.6499	0.0402	0.0555
CE C18:2	-0.7791	1.9492	4.6775	0.1575
CE C18:3	-0.1009	-0.0180	0.0650	0.6656
CE C20:3	-0.1544	-0.0465	0.0613	0.3903
CE C20:4	-1.1645	-0.5701	0.0242	0.0597
CE C20:5	-0.1484	0.0114	0.1712	0.8865
CE C22:5	-0.0067	-0.0017	0.0034	0.5099
CE C22:6	-0.1431	-0.0291	0.0849	0.6103
CE EPA/AA†	-0.0095	0.0168	0.0431	0.2066
CE P/S#	-0.9185	-0.1769	0.5646	0.6338

* A negative result indicates an increase or decrease over time in the reference group and a decrease in the treatment group.

* A positive result indicates an increase or decrease over time in the reference group and an increase in the treatment group.

† Eicosapentaenoic acid to arachidonic acid ratio

Polyunsaturated fatty acid to saturated fatty acid ratio

Table 6.17. Correlation between dietary intake of linoleic acid (C18:2) and eicosapentaenoic acid (C20:5) and the percentage of C18:2 and C20:5 in the plasma triacylglycerols (TAG) and cholesteryl esters (CE) in the treatment periods of Phases 1 and 2

		Red meat diet		Chicken-fish diet	
Dietary fatty acids		**C18:2**	**C20:5**	**C18:2**	**C20:5**
		Phase 1 - Treatment period			
Plasma fatty acids		**Study Group 1**		**Study group 2**	
TAG C18:2	**r**	0.55789	0.0000	0.33867	-0.00729
	p-value	0.0131	1.0000	0.1692	0.9771
TAG C20:5	**r**	-0.35835	0.32475	-0.44387	0.44602
	p-value	0.1319	0.1749	0.0650	0.0636
CE C18:2	**r**	0.49123	0.17213	0.44066	-0.00728
	p-value	0.0327	0.4810	0.0672	0.9771
CE C20:5	**r**	-0.07124	0.34517	0.13946	0.58960
	p-value	0.7720	0.1478	0.5810	0.0100
		Phase 2 - Treatment period			
Plasma fatty acids		**Study Group 2**		**Study Group 1**	
TAG C18:2	**r**	0.14345	0.02337	0.47193	0.35097
	p-value	0.5701	0.9267	0.0413	0.1407
TAG C20:5	**r**	-0.40062	0.21102	0.20299	0.02783
	p-value	0.0994	0.4006	0.4046	0.9100
CE C18:2	**r**	0.20949	-0.02337	0.19474	0.16667
	p-value	0.4041	0.9267	0.4243	0.4953
CE C20:5	**r**	-0.61045	0.39839	0.50022	0.37980
	p-value	0.0071	0.1015	0.0292	0.1087

6.3 Discussion

Fatty acid composition of plasma triacylglycerols and cholesteryl esters

It was shown that two prudent diets, which differed only in the type of "meat" (lean red meat

versus chicken, without skin, and fish), did not result in a significant difference in plasma TC levels of free-living subjects with age-related elevated plasma cholesterol levels (Chapter 5). However, as shown in this chapter, significant differences in the fatty acid composition of plasma TAG and CE were observed as a result of the two treatment diets.

As mentioned in the introduction of this chapter, each of the plasma lipid fractions analysed has a characteristic fatty acid profile (Lindgren, Nichols & Wills, 1961) and is influenced by factors such as diet (Vessby *et al.*, 1980b), age and sex (Holman, Smythe & Johnson, 1979; Botha, 1987), and diseases such as atherosclerosis (Kingsbury *et al.*, 1974). Baseline data also showed that oleic acid predominates in the TAG and linoleic acid in the CE which is characteristic of the fatty acid profile of these lipids (Lindgren, Nichols & Wills, 1961). Compared to the fatty acid composition of TAG and CE of other studies on South African Caucasians (Wolmarans, 1989; Van Staden 1989; Botha, 1987), the percentage of linoleic acid in the TAG and CE found in this study was generally slightly higher or comparable. It is, however, difficult to directly compare the fatty acid composition of TAG and CE of different studies. Studies differ in the number of fatty acids included in the calculations of fatty acid composition of plasma lipids.

Effect of the treatment diets on the fatty acid composition of plasma triacylglycerol and cholesteryl esters

Cross-over analysis showed only significant differences for myristoleic acid (C16:1) and linolenic acid (C18:3n-3) in the TAGs but not in the CEs for the baseline comparisons. No other differences in baseline comparisons or direct-by-period interaction effect were observed for the other fatty acids in the TAGs and CEs. This indicated that there were no first-order or second-order carry-over effects and the analysis for the direct treatment effects could therefore be used.

Saturated fatty acids, MUFAs and PUFAs influence risk factors for CHD differently. Stearic acid in the TAG and CE was significantly higher on the RMD than on the CFD. It is known that SFAs with a carbon number of 12 to 16 increase plasma cholesterol (Keys, Anderson & Grande, 1965b; Hegsted *et al.*, 1965; Denke & Grundy, 1992; Zock, De Vries & Katan, 1994) whereas stearic acid does not (Keys, Anderson & Grande, 1965b; Hegsted *et al.*, 1965; Bonanome & Grundy, 1988; Tholstrup *et al.*, 1994a). *In vitro* studies, however, suggested that

stearic acid might be the most thrombotic of the SFAs (Renaud & Gautheron, 1975; Connor, 1962; Hoak, Warner & Connor, 1967). This was not confirmed by an *in vivo* study in which the effect on 24-hour urinary excretion of TXB_2 and 6-keto $PGF_1\alpha$, the stable metabolites of TXA_2 and PGI_2, was measured (Mustad *et al.*, 1993). Compared to the diet rich in lauric and myristic acids or a typical American diet the diet high in stearic acid did not increase the thrombotic tendency. The difference between the RMD and the CFD in the percentage of stearic acid in the TAG and CE, therefore does not seem to be of importance in terms of the risk factors for CHD.

Monounsaturated fatty acids lower plasma TC when it replaces SFAs in the diet (Mensink & Katan, 1987). Oleic acid was higher in the TAG as well as in the CE on the RMD compared to the CFD. Although the dietary intake of oleic acid is not necessarily reflected in the plasma lipid fractions, oleic acid intake was significantly higher on the RMD than on the CFD. In addition, stearic acid intake was significantly higher on the RMD than on the CFD (Table 4.20) and it is known that stearic acid can be converted to oleic acid in the body (Emken, 1992). The difference observed between the two treatment diets could therefore probably be a reflection of the difference in dietary intake of oleic acid and stearic acid between the RMD and the CFD.

Polyunsaturated fatty acids also lower plasma cholesterol (Goodnight *et al.*, 1982) and it has been shown that n-3 PUFAs decrease platelet aggregation (Goodnight *et al.*, 1982; Kinsella, Lokesh & Stone, 1990) while linoleic acid might also decrease the thrombotic tendency (Goodnight *et al.*, 1982).

The change from a Western type baseline diet with a moderate P/S ratio to a prudent diet with a relatively high P/S ratio of approximately 1.5 was reflected in a higher percentage of linoleic acid in the TAG and the CE on both treatment diets compared to the baseline diet. TAGs reflect recent dietary intake of linoleic acid and the high P/S ratio of the treatment diets was reflected in a significantly higher percentage of linoleic acid in the plasma TAG in the treatment periods compared to the baseline periods. In contrast with TAG, the difference in the percentage of linoleic acid in the CE between the baseline periods and the treatment periods was not significant. This might indicate that the intervention period of six weeks was too short to show an effect of such a small change in the P/S ratio of the diet on the percentage of linoleic

acid in the CE. In addition, the subjects already had a relatively high percentage of linoleic acid in their CE at baseline (57.79 - 59.4%) compared to a value of slightly more than 60% on the prudent diet containing chicken and fish and approximately 59% on the prudent diet containing red meat (Tables 6.4 and 6.5). Tremoli *et al.* (1986) found significant increases in the linoleic acid levels of the TAG and CE after six weeks when the dietary P/S ratio was increased from 0.2 to 2.0. In this study the P/S ratio of the diet at baseline varied between 0.63 and 0.73 while it was 0.93 on the RMD and approximately 1.5 on the CFD (Tables 4.9 and 4.10, Chapter 4). In a study on patients with hyperlipoproteinaemia Vessby *et al.* (1980a) showed that the linoleic acid content of CE was approximately 63% on a diet with a P/S ratio of 1.98 (SD 0.10). Vegetarian diets are also characterised by a high percentage of linoleic acid in the CE and the figures reported were 60.6% (SD 6.25%) for men and 65.06% (SD 4.94%) for women (Melchert *et al.*, 1987). It was also found that a low dietary P/S ratio (0.20 or lower) was associated with a linoleic acid content of below 45% in the CE (Vessby *et al.*, 1980a). In this study the P/S ratio of the diet was moderate and not low during the baseline periods. The percentage of linoleic acid in the CE during these periods varied between 57.79% and 59.4% which is only slightly higher (Wolmarans, 1989) or comparable (Botha, 1987) with figures found in other South African studies. In the study of Wolmarans (1989) the percentage of linoleic acid in the CE varied between 55.5 - 58.3% in men and women aged 20 to 55 years, while in the study of Botha (1987) the figures varied between 56.2 - 59.5% for men and women aged 35 to 55 years.

Although the CFD resulted in a significantly higher P/S ratio than the RMD (Table 4.19), the P/S ratio of the lipid fractions was only significantly higher in the plasma TAG but not in the CE of the CFD compared to the RMD (Tables 6.11 and 6.12). This could indicate that the intervention period was too short to reflect the differences in the P/S ratio of the diet in the CE.

Although dietary data showed that linoleic acid intake was higher on the CFD than on the RMD, the difference was not significant (Chapter 4). Nevertheless, the results of the direct treatment effect showed that linoleic acid in the TAG and the CE, which is a reflection of dietary intake, was significantly higher on the CFD than on the RMD. There are indications in the literature of a negative relationship between total cholesterol as well as LDL-C and the percentage of linoleic acid in the CE (Nikkari *et al.*, 1983a). An inverse relationship between linoleic acid and CHD has been shown (Logan *et al.*, 1978), and significant lower levels of linoleic acid in

platelets and adipose tissue of subjects with angina or first acute myocardial infarction than in controls were reported by Wood *et al.* (1987). There is also concern, however, about the possible role of linoleic acid in the development of atherosclerosis (Hodgson *et al.*, 1993; Felton *et al.*, 1994). A positive association between linoleic acid in the serum and the aortic plaque was found by Felton *et al.* (1994) and they suggested that this implies a direct influence of dietary PUFAs on aortic plaque formation. Other researchers (Leaf, 1995; Truswell, 1995b), however, did not agree with them. Linoleic acid is the major PUFA in aortic plaque (Truswell, 1995b) and it is suggested that fatty acids are highly correlated among various lipid pools in the body (Leaf, 1995). In addition, in LDL, more linoleic acid than any other fatty acid is esterified to cholesterol, and lipid in plaques is derived from LDL (Truswell, 1995b).

Since the fatty acid composition of plasma lipid fractions is not only influenced by diet but also by other factors such as age (Holman, Smythe & Johnson, 1979) and disease (Kingsbury *et al.*, 1974), correlations between dietary intake of fatty acids and their presence in the plasma fractions TAG and CE should be interpreted with care. Linoleic acid is an essential fatty acid and cannot be synthesized in the body although it has been hypothesized that arachidonic acid can be retroconverted to linoleic acid for the reproduction of arachidonic acid (Dupont, 1990). The linoleic acid content of the plasma lipids is therefore a reflection of dietary intake of linoleic acid. A positive correlation between dietary intake of linoleic acid (in g) and the percentage of linoleic acid in the plasma TAG and CE was found, but the correlations were only significant for Study Group 1 (Table 6.17). It is difficult to explain why the correlations were only significant for Study Group 1 and not also for Study Group 2. In Chapter 4, however, there are indications that Study Group 1 complied better with the dietary prescriptions than Study Group 2.

The mean EPA (%) composition of the TAG at baseline varied between 0.21 (SD 0.10) and 0.45 (SD 0.45) which is in agreement with the values of 0.37 (SD 0.41) and 0.43 (SD 0.96) found in another South African study on men and women aged 22 to 45 years who followed a Western type diet (Wolmarans, 1989). The EPA (%) composition of the CE varied between 0.35 (SD 0.20) and 0.56 (SD 0.28) which is lower than the figures of 0.64 (SD 0.46) and 0.70 (SD 0.56) found in the study of Wolmarans (1989).

One way of testing dietary compliance is to use a biomarker. In this study EPA could be used

as a biomarker for dietary compliance because fish, the main source of EPA in the Western type diet, was excluded from the RMD. Plasma phospholipid EPA reflects fish intake better than DHA (Bønaa, Bjerve & Nordøy, 1992) but for long-term habitual intake of fish DHA is a better biomarker (Marckmann *et al.*, 1995). Very small percentages of EPA were observed in the plasma TAG and CE of subjects consuming the RMD who were expected to exclude fish from their diet. Although the main source of EPA in the Western type diet is fish (Nestel, 1987; Simopoulos, 1988), EPA can be synthesized in the body from α-linolenic acid through desaturation and elongation but it is a slow process (Simopoulos, 1988). Dietary data reported in Chapter 4 indicates that α-linolenic intake varied between 0.48 g and 0.54 g on the RMD although this figure might be an underreporting of α-linolenic acid intake as a result of incomplete food composition data (Gouws & Langenhoven, 1986b). Some of the subjects also indicated that they had violated the prescription not to eat chicken or fish during the RMD but the frequency of transgression was limited (Chapter 4). The decrease of EPA in the plasma CE and TAG on the RMD and the increase on the CFD, however, indicated that the dietary instructions were followed. In addition, the percentage of EPA, an n-3 PUFA, in the TAG and the CE was significantly higher on the CFD than on the RMD.

In this study fish was included only twice per week on the CFD and even this limited number of fish meals resulted in a significant difference in the EPA content of the plasma lipids between the two treatment diets. The fish consisted of one portion of hake and alternately one portion of tinned tuna or pilchards in tomato sauce per week which resulted in a mean difference of 0.05 g EPA between the RMD and the CFD (Table 4.20). The study of Bønaa, Bjerve & Nordøy (1992) showed that the fatty acid concentration of phospholipids may be modified by an intake of one dish (or less) of fish per week. Small amounts of fish in the diet therefore seem to have biological effects. Kromhout, Bosschieter & Coulander, (1985) showed that men who consumed two or more fish meals per week had a lower mortality from CHD than those who did not eat fish. EPA has an anti-aggregatory effect compared to arachidonic acid (Laposata, 1995; Nestel, 1987) but there are still many unanswered questions about the advantageous effect of n-3 fatty acids on cardiovascular disease (Endres *et al.*, 1995). A study on two separate but genetically comparable Icelandic populations, however, indicated that n-3 PUFA may be cardioprotective even in an otherwise atherogenic diet (Skúladóttir *et al.*, 1995).

The EPA/AA ratio in plasma free fatty acids might be a coronary risk indicator. It was shown

to be significantly lower in effort angina patients than in controls (Kondo *et al.*, 1986). In the TAG and the CE the ratio of EPA/AA was higher on the CFD than on the RMD and might indicate that the CFD is more beneficial than the RMD in terms of this coronary risk factor. Arachidonic acid is one of the main fatty acids in CE but not in TAG (Lindgren, Nichols & Wills, 1961) and could explain why a significant difference between the RMD and the CFD could be observed in the CE but not in the TAG.

Treatment diets versus reference diet

Differences in the fatty acid composition of the CE between the reference diet and the treatment diets indicated that the reference group consumed more fish than the RMD group but did not consume more fish than the CFD group. The P/S ratio in the TAG, but not in the CE, was significantly higher on the CFD than on the reference diet reflecting the prudent diet composition of the CFD. A possible reason for not observing a significant difference in the CE might be the duration of the experimental period. Six weeks do not seem to be long enough to detect a relatively small change in the P/S ratio of the diet.

6.4 Conclusions

* Differences in the fatty acid composition of the two treatment diets (RMD versus CFD) were reflected in the fatty acid composition of the plasma TAG and CE.

* The linoleic acid and EPA content of the plasma CE was significantly higher on the CFD than on the RMD and this indicates a more favourable lipid profile in terms of the prevention of CHD with the CFD. In addition, a low EPA/AA ratio might be an indicator for the risk of CHD but it was significantly higher and therefore more beneficial on the CFD than on the RMD.

* The P/S ratio of the plasma CE was not affected by the small change in the dietary P/S ratio. This may indicate that an intervention period of six weeks is not long enough to reflect a small change in the dietary P/S ratio in the plasma CE.

* In this study EPA in plasma TAG and CE could be used successfully as a biomarker

to test medium-term dietary compliance of fish intake.

* This study demonstrated that even as few as two fish meals per week resulted in a significantly higher EPA content in the plasma TAG and CE on the CFD than on the RMD.

* The two treatment diets did not result in significant differences in plasma cholesterol levels (Chapter 5). Nevertheless, the effect of the two treatment diets on the fatty acid composition of the plasma TAG and CE indicates a more favourable effect for the CFD than for the RMD in terms of the prevention of CHD.

* The wash-out period of this study seemed to be long enough and with the exception of myristoleic and linolenic acids in the TAGs, no first-order or second-order carry-over effects were observed for the fatty acids in the TAGs and CEs.

CHAPTER 7

SUMMARY OF STUDY DESIGN, RESULTS, CONCLUSIONS AND RECOMMENDATIONS

7.1 Design of the study

This clinical trial had a cross-over design and the two phases each consisted of a baseline (Phase 1, three weeks; Phase 2, one week), a treatment (six weeks) and a post-treatment (six weeks) period with a washout period of approximately two months in-between. Seventy subjects were recruited for the study. Subjects were matched for age and plasma TC levels and randomly allocated to one of three study groups. Study Groups 1 (n = 28) and 2 (n = 28) were the treatment groups while Study Group 3 (n = 14) was the reference group. The treatment groups followed the treatment diets for six weeks in each phase. Study Group 1 followed a prudent diet which contained lean red meat, no chicken and fish, as the only "meat" in the diet in Phase 1, while Study Group 2 followed a prudent diet containing chicken and fish as the only "meat" in the diet and no red meat in Phase 1. The diets of Study Groups 1 and 2 were crossed over in Phase 2. Study Group 3 was the reference group and the habitual diet was followed throughout the study. Fifty-two subjects completed the cross-over study (Study Group 1, n = 21; Study Group 2, n = 18; Study Group 3, n = 13), but dietary results are reported for only 50 subjects.

Subjects received pre-packed lean red meat, chicken (without skin) and fish portions to provide for the number of "meat" exchanges prescribed. The subjects were responsible for the remainder of the food exchanges prescribed i.e. milk, bread, fruit and vegetables, and fat.

Blood samples were collected after an overnight fast, for the analyses of TC, TAG, LDL_1-C, LDL_2-C, HDL-C, HDL3-C, VLDL-C and VLDL-TAG. The fatty acid composition of plasma TAG and CE were also determined. Other measurements taken were blood pressure, body weight and height. Seven-day weighed and estimated dietary records were kept during the baseline and treatment periods.

The main aim of the study was to investigate the effect of two prudent diets, which differed only in the type of "meat" (lean red meat versus chicken, without skin, and fish), on plasma lipids and lipoproteins of free-living subjects with age-related elevated plasma TC levels. Specific objectives were to evaluate dietary compliance; to report on dietary intake; and to evaluate the effect of the treatment diets on the fatty acid composition of plasma TAGs and CEs.

7.2 Results

7.2.1 Dietary intake

7.2.1.1 Dietary compliance

Results showed that mean energy intakes, as determined by the seven-day dietary records, were significantly lower than the energy prescribed. The differences between the mean energy intakes in the baseline periods and the treatment periods were also significantly different except for Study Group 2 in the sixth week of the treatment period of Phase 2.

Dietary compliance was measured by analysing the seven-day dietary records for the number of food exchanges consumed. The mean portion of chicken plus fish consumed was approximately 5 g smaller than the lean red meat portion. Although the number of meat exchanges consumed was slightly higher than the number prescribed, the difference was only significant in Study Group 1 in the second but not the sixth week of the treatment period.

Evaluation of dietary compliance by means of a questionnaire showed that 97.1% of the subjects on the treatment diets were of the opinion that they complied 70% or more with the dietary prescription. Non-compliance with regard to the consumption of alcohol was only 8.6%.

Dietary compliance seemed to be better in Phase 1 than in Phase 2. Subjects violated the prescriptions not to eat lean red meat or chicken and fish when it was not prescribed, but the majority who transgressed only did so twice or fewer times during the six-weeks of the treatment periods.

7.2.1.2 Energy and macronutrient intakes

(a) ***Intakes of Study Groups 1, 2 and 3 in the baseline and treatment periods***

Since the percentage of energy provided by chicken and fish was lower than the percentage of energy provided by red meat, it could explain why energy intake tended to be lower on the CFD than on the RMD in Study Groups 1 and 2.

In Study Groups 1 and 2 dietary intervention resulted in a decrease in energy intake, and total fat intake decreased to less than 30%E except in Study Group 2 (31%E) on the RMD. Saturated fat intake decreased to less than 10%E on the RMD and CFD but was lower on the latter. Both treatment diets resulted in a higher P/S ratio than at baseline in Study Groups 1 and 2 but the P/S ratio was higher on the CFD (approximately 1.5) than on the RMD (0.93). Dietary cholesterol intakes were higher on the baseline diet than on the RMD and CFD diets. The Keys dietary score, which is an indication of the atherogenicity of the diet, was lower on the RMD and the CFD than at baseline and also lower on the CFD than on the RMD in both study groups. On the RMD the Keys dietary score varied between 28 and 30.2 while it varied between 19.1 and 22.1 on the CFD.

In the reference group, Study Group 3, dietary intake remained more or less stable throughout the study and met the criteria for a Western diet. Total fat intake was more than 30%E, saturated fat intake was approximately 11%E, the P/S ratio of the diet varied between 0.64 and 0.70 during the study, and the dietary cholesterol intake was approximately 305 mg. The Keys dietary score on the habitual diet of Study Group 3 was very similar to the Keys dietary scores on the baseline diets of Study Groups 1 and 2.

(b) ***Differences between the baseline diet and the red meat or chicken-fish diets***

Dietary intervention resulted in significantly lower energy, total fat, saturated fat, monounsaturated fat and dietary cholesterol intakes on the RMD and the CFD than on the baseline diets. Protein and carbohydrate intakes, and the P/S ratio of the diet were, however, significantly higher on the treatment diets than on the baseline diets while fibre intake was also significantly higher on the CFD than on the baseline diet. Alcohol intake did not differ significantly between the baseline and the treatment diets.

(c) *Cross-over analysis*

There were no first order carry-over (baseline comparisons) or second order carry-over (direct-by-period interaction) effects for the energy and macronutrient intake, the Keys dietary score and the dietary P/S ratio in Study Groups 1 and 2.

(d) **Direct treatment effect**

The direct treatment effect refers to the effect of the RMD and the CFD within the cross-over design. Total energy, total fat, saturated fat, monounsaturated fat and dietary cholesterol intakes as well as the Keys dietary scores were significantly higher on the RMD than on the CFD. Carbohydrate and polyunsaturated fat intake and the P/S ratio of the diet were significantly higher on the CFD than on the RMD. Protein, fibre and alcohol intake did not differ between the two treatment diets.

(e) ***Treatment diets versus the reference diet***

Results showed significant differences between the changes in the RMD and the reference diet and between the CFD and the reference diet. Significant differences were observed for energy, total fat, saturated fat, monounsaturated fat, dietary cholesterol and the Keys dietary score. All these differences between the means were negative indicating a decrease over time in the treatment groups. The differences between the means were positive for carbohydrate and the P/S ratio for the RMD and the CFD versus the reference diet, while it was also positive for protein in the CFD versus the reference diet. These positive differences between means indicated an increase over time in the treatment diets.

7.2.1.3 Fatty acid intakes

(a) ***Intakes of Study Groups 1 and 2 in the baseline and treatment periods***

The decrease in total and saturated fat intake in Study Groups 1 and 2 was reflected in the consumption of the individual fatty acids. Oleic acid intake also decreased markedly on the RMD and CFD. Linoleic acid intake decreased on the RMD and CFD diet compared to the baseline diet but this tendency was not consistent. In Study Groups 1 and 2 results showed an increased intake of EPA on the CFD and a decrease on the RMD.

In Study Group 3 the intake of myristic (C14:0), palmitic (C16:0) and oleic (C18:1) acids did not show major changes during the study. The mean intake of EPA by Study Group 3 during

the study agreed with the mean intakes of Study Groups 1 and 2 during the baseline periods.

(b) *Results of baseline diet versus the red meat and chicken-fish diets*

The intake of the SFAs acids lauric, myristic and palmitic acids, was significantly higher on the baseline diet than on the RMD or the CFD. Dietary manipulations also resulted in a significantly lower intake of the monounsaturated fatty acid, oleic acid, on the treatment diets. Linoleic acid intake did not differ between the baseline and treatment diets. The intake of EPA was significantly lower on the RMD but significantly higher on the CFD than on the baseline diet.

(c) *Cross-over analysis*

There were no first order carry-over (baseline comparisons) or second order carry-over (direct-by-period interaction) effects for fatty acid intakes in Study Groups 1 and 2.

(d) *Direct treatment effect*

The RMD resulted in a significantly higher intake of the SFAs myristic, palmitic and stearic acids, but also of the MUFAs myristoleic and oleic acids than on the CFD. The intake of the long chain PUFAs C20:5, C22:5 and C22:6, was significantly higher on the CFD than on the RMD.

(e) *Treatment diets versus the reference diet*

Significant differences in myristic, palmitic, stearic and oleic acids were observed for changes in the RMD and the CFD versus the reference diet. These differences were negative indicating a decrease over time in the treatment diet. Significant differences were also observed for C22:5 and C22:6 but not for C20:5 ($p = 0.0516$) in the CFD, but these differences were positive indicating an increase over time in the CFD.

7.2.2 Plasma lipids and lipoproteins, blood pressure and Keys equation

7.2.2.1 Values at baseline and in the treatment periods for Study Groups 1, 2 and 3

Although everybody, with the exception of one subject, had age-dependent elevated plasma TC levels at recruitment, the mean plasma TC levels were not high and were less than 5.6 mmol/L in all three study groups. At each measuring point the mean TAG levels of Study Group 3 were

higher than those of Study Groups 1 and 2.

Dietary intervention was reflected in the changes in plasma lipids and lipoproteins, but lipoprotein levels showed a tendency to remain relatively constant in Study Group 3 which was the reference group.

7.2.2.2 Baseline diet versus the red meat diet and the chicken-fish diet

Significant differences were observed between the baseline and treatment periods. On both treatment diets TC, HDL-C:TC, LDL_1-C, LDL_2-C, LDL_1-apo B and LDL_2-apo B were significantly lower on the treatment diets than on the baseline diet. Body mass was also significantly different on the CFD than on the baseline diet, while diastolic blood pressure was significantly lower on the RMD than on the baseline diet.

7.2.2.3 Cross-over analysis

Baseline differences were only observed for LDL_2-apo B between Study Group 2 and Study Group 3. The only difference between the baseline values of Study Group 1 and Study Group 2 was for VLDL-TAG.

There were, however, no other significant differences for baseline comparisons or for the direct-by-period interaction effect for body mass, plasma lipids and lipoproteins, and blood pressure in Study Groups 1 and 2.

7.2.2.4 Direct treatment effect

The only significant differences between the RMD and the CFD were a lower HDL-C level and lower diastolic blood pressure on the CFD. Body mass did not differ significantly between the two treatment diets.

7.2.2.5 Treatment diets versus reference diet

There was no significant difference between the changes in the RMD and the reference diet for body mass, plasma lipids and lipoproteins, and blood pressure. Significant differences between the changes on the CFD and the reference diet for body mass, LDL_2-C and LDL_2-apo B were found. These differences were negative which indicated a decrease over time on the CFD.

7.2.2.6 Plasma total cholesterol versus age-dependent action limits for the treatment of hypercholesterolaemia

Results showed a decrease of plasma TC levels at baseline before dietary intervention was introduced. A marked shift of subjects to the low-risk category for plasma TC was observed on the RMD and the CFD. At the end of the post-treatment period there was a tendency for plasma TC levels to shift back to elevated levels.

7.2.2.7 Use of the Keys equation to predict a change in plasma total cholesterol in response to a change in dietary fat and cholesterol intake

Different combinations of SFAs and PUFAs were used in the Keys equation and a good agreement between the predicted mean and the measured mean change in plasma TC between the baseline and treatment diets was observed when stearic acid formed part of the total SFAs or was included with myristic and palmitic acids in the formula.

There was a significant difference between the predicted and measured change in plasma TC between the baseline diet and the CFD when total fat and total saturated fat or the saturated fatty acids lauric, myristic and palmitic acids, and total polyunsaturated fatty acids were used in the Keys equation.

7.2.3 Fatty acid composition of plasma triaclyglycerol and cholesteryl ester

7.2.3.1 Values at baseline and in the treatment periods for Study Groups 1, 2 and 3

The exclusion of fish from the RMD diet in Study Groups 1 and 2 was reflected in the percentage of EPA in the plasma TAG and CE and was more pronounced in the latter. In Study Group 3 the EPA content of the plasma TAG and CE tended to be higher than in the baseline periods of Study Groups 1 and 2 and even higher than on the CFD.

The percentage oleic acid in the plasma TAG decreased on the CFD in Study Groups 1 and 2. In Study Groups 1 and 2 the percentage of linoleic acid in the plasma TAG increased on the RMD and the CFD but the increase seems to be more pronounced on the CFD. In Study Group 3 linoleic acid content of the plasma TAG remained more or less constant.

As a result of the high P/S ratio of the CFD the linoleic acid content of the CEs was

approximately 60%.

7.2.3.2 Baseline diet versus the red meat diet and the chicken-fish diet

The percentage of oleic acid was significantly lower in the TAG as well as the CE on the RMD and the CFD compared to the baseline diet. Compared to the baseline diet, the percentage of stearic acid in the plasma TAG and CE was lower on the RMD. The stearic acid content of the CE but not the TAG was also lower on the CFD than on the baseline diet. The percentage of linoleic acid in the plasma TAGs was significantly higher on the RMD and the CFD compared to the baseline diet but no difference was observed in the CE.

There was no significant difference in the EPA content of the plasma TAG or CE between the baseline and treatment diets.

7.2.3.3 Cross-over analysis

There was a significant difference in the baseline comparisons of the palmitoleic acid and linolenic acid content of the plasma TAG of Study Groups 1 and 2. No other significant differences in the plasma TAG or CE were observed for the baseline comparisons or for the direct-by-period interaction effect.

7.2.3.4 Direct treatment effect

In the plasma TAG and CE the percentage of stearic acid and oleic acid was significantly higher on the RMD than on the CFD. The percentage of linoleic acid, EPA, docosapentaenoic acid, and DHA, and the EPA/AA ratio in the plasma TAG and CE were significantly lower on the RMD than on the CFD. The P/S ratio of the plasma TAG was significantly lower on the RMD than on the CFD but no difference was observed for the CE.

7.2.3.5 Treatment diets versus reference diet

The only significant difference in the fatty acid composition of the plasma TAG observed between the change in the RMD and the reference diet was in the percentage of DHA in the plasma TAG. This difference was negative indicating a decrease over time on the RMD. Significant differences between the changes in the chicken-fish diet and the reference diet were observed for palmitic and linoleic acids and for the P/S ratio of plasma TAG. The difference for palmitic acid was negative while it was positive for linoleic acid and the P/S ratio.

In the CEs significant differences between the changes on the RMD and the reference diet were observed for the percentage of EPA and DHA and the EPA/AA ratio in the CE. These differences were negative.

7.3 Conclusions

7.3.1 Dietary compliance

* Non-compliance with the dietary prescriptions did not seem to be a major problem in this study on free-living subjects. Transgression of the recommendations for the consumption of lean red meat, chicken and fish was limited to only a few episodes. In addition, the majority of subjects indicated that they followed the dietary prescriptions well. Despite the fact that the reference group knew they had hypercholesterolaemia, their diet remained more or less the same throughout the study which indicated that they followed the instruction not to change their diet. Although it is known that studies on free-living subjects are less rigidly controlled than those in metabolic wards, non-compliance was limited and should not have influenced the outcome of the study.

* Since fish is the main source of EPA in the diet and chicken also contains a small amount of EPA, it could be used successfully as a biomarker for dietary compliance on the RMD. The significant difference in EPA intake between the RMD and the CFD and the significant differences in the percentage of EPA in the plasma TAG and the CE between the RMD and the CFD confirmed dietary compliance.

7.3.2 Effect of two prudent diets, containing either lean red meat or chicken and fish, on plasma lipids and lipoproteins

* The placebo effect or the knowledge of an elevated plasma TC level has an influence on the plasma TC level. Plasma TC levels decreased even before dietary intervention was introduced. This decrease could be ascribed to the placebo effect or because subjects learned at recruitment that they had elevated plasma TC levels.

* This study showed that it is possible to change a Western diet to a prudent diet within six weeks, but specific guidelines and intensive dietary intervention are needed to achieve this goal.

* The favourable effect of a prudent diet compared to a Western type diet on the lipid profile of humans was confirmed. Plasma TC, LDL_1-C, LDL_2-C, LDL_1-apo B and LDL_2-apo B were significantly lower on the prudent diet than on the Western-type baseline diet irrespective of whether the prudent diet contained lean red meat or chicken (without skin) and fish. It was also shown that the prudent diet which resulted in a decrease in fat and increase in carbohydrate did not necessarily decrease HDL-C or increase TAG levels.

* The significant differences in dietary intake between the RMD and the CFD, the difference in the Keys dietary score and the Keys equation indicated that the RMD was more atherogenic than the CFD. This, however, was not confirmed by analysis for the direct treatment effect. The atherogenic lipoproteins did not differ significantly between the RMD and the CFD.

* This study confirmed that total fat, saturated fat and dietary cholesterol influence HDL-C levels. Total fat, saturated fat and dietary cholesterol intakes were significantly higher on the RMD than on the CFD and could thus explain the significantly higher level of HDL-C on the RMD than on the CFD.

* When subjects follow a mixed diet consisting of ordinary food the Keys equation seems to be effective in predicting a change in plasma TC levels in response to a change in total fat and dietary cholesterol intakes.

* Although there were significant differences in dietary intake between the diet of the reference group and the RMD, these were too small to result in significant differences in the lipid profiles. Differences in energy and total fat intakes between the diet of the reference group and the CFD were significant and seemed to be large enough to result in a significant difference in body mass, LDL_2-C and LDL_2-apo B between the two groups.

7.3.3 Effect of two prudent diets, containing either lean red meat or chicken and fish, on plasma fatty acid composition of plasma triacylglycerol and cholesteryl ester

* A small difference in the intake of EPA can influence the fatty acid composition of the plasma. A difference of 0.05 g in EPA intake resulted in a significantly higher percentage of EPA in the plasma TAG and CE on the CFD than on the RMD.

* There are indications that more than six weeks are needed for a small change in the P/S ratio of the diet to reflect a difference in the P/S ratio of the CE. Although there was a significant difference between the RMD and the CFD in the P/S ratio of the TAGs, this difference was not observed in the CE.

* The higher EPA/AA ratio in the TAG and CE on the CFD than on the RMD may indicate that the CFD is more favourable in terms of CHD than the RMD.

* The significantly higher EPA content of the TAG and CE on the CFD than on the RMD could explain the significantly lower diastolic blood pressure on the CFD than on the RMD.

As a general conclusion, this study did not show a difference in atherogenic lipoproteins when extremes were tested, i.e. a daily portion (seven days per week) of lean red meat versus chicken, without skin (five times per week), or fish (twice per week) included within the framework of a prudent diet. It could therefore be concluded that the replacement of some of the lean red meat portions in the prudent diet will not necessarily improve the lipid profile further. However, the inclusion of fish could influence the EPA content of the fatty acids in the plasma and this may have implications in terms of improved blood pressure and thrombogenesis.

7.4 Recommendations

* This study confirmed the findings of others and indicated that lean red meat can be included in the diet of those with hypercholesterolaemia. This should only be done against the background of a prudent diet. Depending on energy needs (7 500 to 14 000 kJ), a daily portion of lean red meat varying between 120 g and 210 g, can be included in a lipid-lowering diet.

It is recommended that:
specific quantitative recommendations regarding the inclusion of lean red meat in the diet are included in the dietary guidelines for those with hyperlipidaemia. This could help combat the misconception that red meat should be excluded from a lipid-lowering diet.

* A small difference between the RMD and the CFD was reflected in EPA content as well as the EPA/AA ratio of the TAG and the CE and probably also had a beneficial effect in terms of diastolic blood pressure.

It is recommended that:
fish, which is the main source of EPA in the Western diet, is included in the lipid-lowering diet. At least two fish meals per week, of which at least one is from a medium fat fish, should be encouraged.

* The impact of the difference in the EPA content of the TAG and CE between the RMD and the CFD may be important in terms of its impact on the n-6/n-3 ratio and thrombogenesis. On the prudent diet the intake of linoleic acid usually increases and this has implications for the 18:2/18:3 ratio.

It is recommended that:
the impact of the small difference in EPA intake between the two treatment diets on thrombogenesis be investigated.

* There are still many unanswered questions about the relationship between the intake

of red meat and colon and rectum cancer. The replacement of red meat with poultry and fish did not emerge as a protective factor in the study of Bidoli *et al.* (1992) while in another study it did (Giovannucci *et al.*, 1992). It is not clear whether the association between red meat intake and colorectal cancer is due to the fat content of red meat.

It is recommended that:

epidemiological studies in which the relationship between the intake of red meat and colo-rectal cancer is investigated, distinguish between lean red meat and fatty red meat.

Final comment and recommendation

A large part of the South African population is a population in transition. Economic empowerment of previously marginalised South Africans could in future lead to changes in dietary intake. These changes might include an increase in the consumption of animal products, e.g. red meat, with a concomitant increase in the consumption of total fat, especially saturated fat. This increase in total fat and saturated fat intake could contribute to overweight and hypercholesterolaemia and result in the development of the diseases of lifestyle. Therefore, to combat the development of the diseases of lifestyle, it is recommended that the South African consumers be encouraged to eat lean red meat instead of fatty red meat irrespective of whether they have hypercholesterolaemia or not.

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ADDENDUMS

ADDENDUM A

25 Junie 1990

90/10/2105

Geagte mnr/mev/mej

NAVORSINGSPROJEK BY DIE NAVORSINGSINSTITUUT VIR VOEDING-SIEKTES

Die Navorsingsinstituut vir Voedingsiektes van die Mediese Navorsingsraad beplan 'n navorsingsprojek om vas te stel of rooivleis (skaap- en beesvleis) met hoender en vis vervang moet word om die bloedcholesterolvlak te beheer.

Ons soek vrywilligers (mans en vrouens tussen die ouderdom van 20 en 55 jaar) om aan hierdie projek deel te neem. Graag neem ons die vrymoedigheid om u te nader vir deelname aan die projek.

1. U sal op grond van u ouderdom, bloedcholesterolvlak en gewig gekeur word vir die studie. Ons soek vrywilligers wat geen medikasie gebruik nie.

2. Die projek sal teen ongeveer Augustus 1990 begin en teen die middel van Mei 1991 eindig.

* Die projek sal uit 2 fases bestaan, elk met 'n

   (a) 3 weke voor-eksperimentele periode (basislyn periode)
   (b) 6 weke eksperimentele periode
   (c) 6 weke na-eksperimentele periode.

   Gedurende Desember 1990 en Januarie 1991 sal geen ondersoeke op die vrywilligers gedoen word nie (uitwasperiode).

3. U sal 'n mediese ondersoek ondergaan.

4. By 6 geleenthede tydens die studie sal u vir 7 dae alles wat u eet en drink moet neerskryf.

5. 'n Spesiale dieet (nie 'n verslankingsdieet) sal vir u uitgewerk word.

6. Almal sal tydens die basislyn-, na-eksperimentele- en uitwasperiode hulle normale dieet volg. Na die drie weke basislyn sal die proefpersone vir 6 weke in een van die volgende 3 groepe val:

(a) spesiale dieet met rooivleis (skaap- en beesvleis) as die "enigste vleis" in die dieet.

(b) spesiale dieet met hoender en vis as die "enigste vleis" in die dieet.

(c) U volg u normale dieet.

7. Spesiale rooivleis-, vis- en hoenderporsies sal gratis aan u verskaf word.

8. U sal bereid moet wees om by ongeveer 9 geleenthede in elke fase, buite werktyd (d.w.s 07:00 - 8:30 of na 16:45), beskikbaar te wees vir die neem van bloedmonsters, dieetinstruksies en die uitreiking van die vleis/vis/hoender porsies.

9. Indien u 'n getroude man is, sal ons graag u vrou wil ontmoet voordat die eksperimentele periode begin om die dieet aan haar te verduidelik.

10. Hou asseblief in gedagte dat u deelname aan die studie benadeel mag word indien u dikwels uitstedig is.

Die studie is goedgekeur deur die Etiese Komitee van die Mediese Navorsingsraad en daar sal onder alle omstandighede aan die etiese standaarde voldoen word soos voorgeskryf.

Dit sal vir ons 'n voorreg wees om u as proefpersoon te kan gebruik. Hierdie navorsing kan 'n belangrike bydrae maak tot die gesondheid van mense wat hulle bloedcholesterol wil beheer en ook om die voorkoms van hartaanvalle te verminder.

Vul asseblief die aangehegde strokie in en dui aan of u belangstel/nie belangstel om aan die projek deel te neem. Indien u belangstel om deel te neem, sal ons met u in verbinding tree. Vir enige verdere inligting kan u met mej P Wolmarans by 9320311 x 268 skakel.

Ons hoor graag van u nie later nie as 9 Julie 1990.

Vriendelike groete

MEJ P WOLMARANS
PROGRAM: VOEDING

PW/ean

Prof, Dr, Mnr ..... stel belang/nie belang (skrap wat nie van toepassing), om aan die navorsingsprojek by die Navorsingsinstituut vir Voedingsiektes deel te neem.

Telefoonnommer .....

Handtekening .....

(Stuur aseblief terug na: Dr JA van der Merwe
Hoof Mediese Amptenaar
SANLAM HOOFKANTOOR

Sny hier

ADDENDUM B

MEDIESE NAVORSINGSRAAD - NAVORSINGSINSTITUUT VIR VOEDINGSIEKTES

Risikovraelys

Projek 90102105

Kodenommer [ ][ ][ ][ ] 4

ALGEMENE INLIGTING

1. Manlik = 1 Vroulik = 2 [ ] 5

2. Huwelikstaat

   (a) Nooit getroud/was getroud 1

   (b) Is getroud/woon saam [2] [ ] 6

3. Ouderdom met <u>laaste</u> verjaardag [ ][ ] 8

4. Wat is u hoogste onderwyskwalifikasie

   < st 1 1
   st 1 2
   st 2 3
   st 3 4
   st 4 5
   st 5 6
   st 6 7
   st 7 8
   st 8 9
   st 9 10
   st 10 11
   st 10 + 1 jaar hoër onderrig 12
   st 10 + 2 jaar hoër onderrig 13
   st 10 + 3 jaar hoër onderrig 14
   st 10 + 4 jaar of meer hoër onderrig [15] [ ][ ] 10

5. Watter soort werk doen u ..... [ ][ ] 12
   .....

6. Wie is u werkgewer ..... [ ][ ] 14
   .....

7. Watter taal gebruik u meestal by die huis?

Afrikaans	1	
Engels	2	
Afrikaans en Engels	3	
Ander (noem):	4	15

FAMILIE GESKIEDENIS

8. Het enige van die volgende koronêre hartsiekte?

	Ja	Nee	Weet nie	NVT	
(a) U eie moeder	1	2	3	4	
(b) U eie vader	1	2	3	4	
(c) U eie broer(s)	1	2	3	4	
(d) U eie suster(s)	1	2	3	4	19

9. Het enige van die volgende koronêre hartsiekte gehad voor die ouderdom van 50 jaar?

	Ja	Nee	Weet nie	NVT	
(a) U eie moeder	1	2	3	4	
(b) U eie vader	1	2	3	4	
(c) U eie broer(s)	1	2	3	4	
(d) U eie suster(s)	1	2	3	4	23

10. Het enige van die volgende diabetes mellitus?

	Ja	Nee	Weet nie	NVT	
(a) U eie moeder	1	2	3	4	
(b) U eie vader	1	2	3	4	
(c) U eie broer(s)	1	2	3	4	
(d) U eie suster(s)	1	2	3	4	27

11. Het enige van die volgende hoë bloeddruk?

	Ja	Nee	Weet nie	NVT	
(a) U eie moeder	1	2	3	4	
(b) U eie vader	1	2	3	4	
(c) U eie broer(s)	1	2	3	4	
(d) U eie suster(s)	1	2	3	4	31

12. Het enige van die volgende hoë bloedcholesterol?

	Ja	Nee	Weet nie	NVT	
(a) U eie moeder	1	2	3	4	
(b) U eie vader	1	2	3	4	
(c) U eie broer(s)	1	2	3	4	
(d) U eie suster(s)	1	2	3	4	35

13. Het enige van die volgende beroerte gehad?

	Ja	Nee	Weet nie	NVT	
(a) U eie moeder	1	2	3	4	
(b) U eie vader	1	2	3	4	
(c) U eie broer(s)	1	2	3	4	
(d) U eie suster(s)	1	2	3	4	39

14. **ROOK GEWOONTE**

Rook u (JA = 1 Nee = 2)

(a) Nee, het nog nooit gerook nie (gaan na vraag 23)

(b) Het voorheen gerook, maar rook nie meer nie (gaan na vraag 18)

(c) Rook af en toe (minder as een sigaret ens. per dag)

(d) Rook tans 43

15. Wat rook u (JA = 1 NEE = 2 NVT = 0)

(a) sigarette

(b) pyp

(c) sigare 46

16. Sigarette (NVT = 0)

(a) Hoe lank rook u reeds sigarette? . 53

(b) Hoeveel sigarette rook u per dag?

17. Pyp (NVT = 0)

(a) Hoe lank rook u reeds pyp? .

(b) Rook u daagliks pyp? (JA = 1 NEE = 2 NVT = 0) 58

18. Sigare (NVT = 0)

(a) Hoe lank rook u reeds sigare? .

(b) Hoeveel sigare rook u per dag? 64

19. Gewese rokers (NVT = 0)

(a) Hoe lank gelede het u opgehou rook? .

20. Wat het u gerook? (JA = 1 NEE = 2 NVT = 0)

(a) sigarette

(b) pyp

(c) sigare 72

21. Sigarette

(a) Hoeveel jare het u sigarette gerook? [ ][ ].[ ]

(b) Hoeveel sigarette het u per dag gerook? [ ][ ][ ] 79

22. Pyp

(a) Hoeveel jare het u pyp gerook? (NVT = 0) [ ][ ].[ ] 83

23. Sigare

(a) Hoeveel jare het u sigare gerook? (NVT = 0) [ ][ ].[ ]

(b) Hoeveel sigare het u per dag gerook? [ ][ ] 89

FISIEKE AKTIWITEIT

24. Bestaan u werksdag uit:

(a) Min fisieke aktiwiteite (bv sittende kantoorwerk) [1][ ]

(b) Matige fisieke aktiwiteite (bv huiswerk, tuinwerk, loopwerk) [2][ ]

(c) Harde fisieke aktiwiteite (bv harde tuinwerk, bouwerk, houtkap) [3][ ] 92

25. Neem u buite u werksdag deel aan

(a) Geen fisieke aktiwiteite [1][ ]

(b) Matige fisieke aktiwiteite (bv fietsry vir ontspanning, golf, krieket, ligte oefeninge, rolbal, stap, ligte tuinwerk, sosiale tennis) [2][ ]

(c) Harde fisieke aktiwiteite (bv bergklim, aërobiese oefeninge, perdry, tennis, draf, fietsry, hokkie, muurbal) [3][ ]

ALKOHOL INNAME

26. Gebruik u alkohol?

JA = 1 NEE = 2 [ ] 96

27. Indien JA hoeveel keer en hoeveel per keer (ml)? (NVT = 0)

	PD	PW	PM	Hoeveel (ml) per keer
Bier				[ ].[ ][ ][ ]
Brandewyn/whisky/rum				[ ].[ ][ ][ ]
Ander spiritualieë				[ ].[ ][ ][ ]
Droëwyn				[ ].[ ][ ][ ]
Soetwyn				[ ].[ ][ ][ ]
Likeur				[ ].[ ][ ][ ]
Ander				[ ].[ ][ ][ ]

..... 124

DIEETINLIGTING

28. Het u al ooit 'n dieet vir die beheer van u bloedcholesterol ontvang? (JA = 1 NEE = 2 NVT = 0) [ ] 125

29. Volg u die dieet? (JA = 1 NEE = 2 NVT = 0) [ ] 126

30. Hoe lank volg u die dieet? (jare en maande) (NVT = 0) [ ][ ].[ ] 130

31. By wie het u die dieet ontvang? (NVT = 0)

Dieetkundige	1 [ ]
Dokter	2 [ ]
Hospitaal	3 [ ]
Ander (noem)	4 [ ] 134

32. Wat behels die dieet wat u volg? (NVT = 0)

(a) eet minder vetterige voedsel	1 [ ]
(b) eet minder voedsel hoog in cholesterol	2 [ ]
(c) eet minder diervette	3 [ ]
(d) eet meer polionversadigde vette (bv sonneblomolie en bakkies margarien)	4 [ ]
(e) eet minder sout	5 [ ]
(f) eet minder in die algemeen	6 [ ]
(g) ander	7 [ ] 141
.....	

33. Hoeveel keer per week eet u

(a) Skaapvleis	[ ][ ].[ ]
(b) Beesvleis (ingeslote boerewors, maalvleis, koue vleis)	[ ][ ].[ ]
(c) Varkvleis (ingeslote varkwors, ham)	[ ][ ].[ ]
(Indien minder as 1 keer per week druk uit as 'n breuk)	153

34. Hoe groot is die porsie rooivleis wat u normaalweg eet?

(a) Skaapvleis	[ ][ ][ ][ ]
(b) Beesvleis	[ ][ ][ ][ ]
(c) Varkvleis	[ ][ ][ ][ ]
(Gebruik voedselmodel en druk uit in gram)	165

35. Hoevel keer per week mag u rooivleis (skaap, bees, vark) eet? (NVT = 0) [ ][ ] 167

36. By wie het u hierdie instruksie gekry? (NVT = 0)

Dieetkundige	[ ]
Dokter	[ ]
Ander (noem .....)	[ ] 170

ADDENDUM C

VOLUNTEER CONSENT FORM

1. I, the undersigned, voluntarily agree to take part in the following study:

   The effect of the type of "meat" in a lipid lowering diet, on lipid metabolism and haematological factors in man.

2. I have been fully informed of the purpose and nature of the study as well as the advantages and possible adverse effects resulting from the undermentioned procedures and/or treatment, as explained on the reverse side of this form and I understand what it says.

3. Since natural food will be used, it is extremely unlikely that any unexpected or unusual symptoms will occur. If anything untoward does occur, I will report this at once.

4. The nature of the procedures and/or treatment to be undertaken is:

   (a) Blood will be drawn

   (b) Fat biopsy (under local anaesthesia)

   (c) Dietary manipulation

5. The procedures will be carried out by medical doctors from the MRC and the treatment and dietary consultations will be carried out by dietitians from the MRC.

6. I understand that I can recall my consent at any time.

Name of volunteer: _____

Postal address: _____

Telephone number: _____ (h) _____ (w)

Signed at: ..... this ..... day of ..... 1990.

....
Volunteer Person who informed volunteer

.....
As witness:

SUMMARY OF INFORMATION GIVEN TO PATIENT

Medical Evaluation: Volunteers will be medically investigated before the start of the study.

Duration of the study: This study will consist of two phases each with:

(a) a three week baseline period
(b) a six week experimental period
(c) a six week post-experimental period

There will be a washout period of approximately two months between phases I and II.

Diet

(a) Habitual diet: During the baseline, post-experimental and washout periods.
(b) Experimental diets: During the experimental periods of phase I and II volunteers will follow specially designed diets.
    (i) lean red meat will be the "only meat" in the diet or
    (ii) chicken and fish will be the "only meat" in the diet

Volunteers will follow diet (i) or (ii) in phase I and will switch over in phase II.

- The red meat, fish and chicken will be supplied to the volunteers during the experimental periods of phase I and II.

- The rest of the diet must be provided by the volunteers themselves.

Dietary records: Dietary records for 7 days will be kept as follows:

(a) once during the baseline period and
(b) twice during the experimental period

- Dietary records will be checked twice during the seven days.

Blood sampling* and other measurements

(a) three times during the baseline period
(b) twice during the experimental period
(c) once during the post-experimental period

Height and weight: Together with the blood sampling

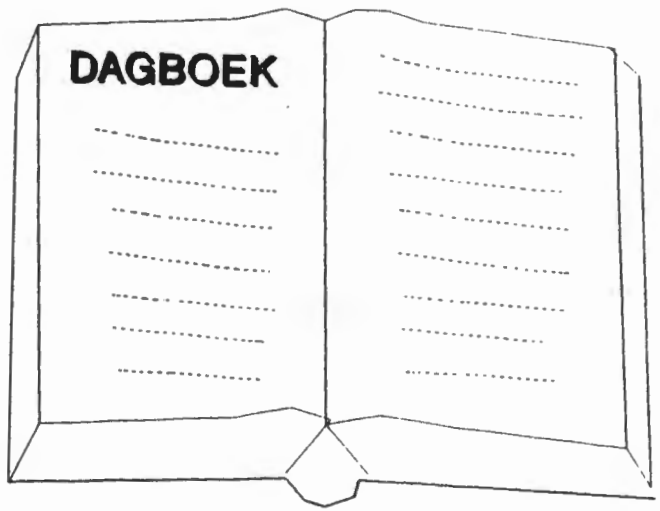
~~Fat biopsy:* Once in each phase under local anaesthesia~~

Blood pressure: During baseline, experimental and post-experimental periods.

~~* These procedures are completely safe although puncturing of the skin is involved. The risk of bleeding and infection, however, exist in both.~~

MNR
MRC

Navorsingsinstituut vir Voedingsiektes
Research Institute for Nutritional Diseases



ADDENDUM D

Baie dankie vir u deelname aan die navorsingsprojek.

In hierdie dagboek vind u die datums waarop u bloed getrek sal word, u inligting sal ontvang oor die hou van 'n dieetrekord, asook die datums waarop die rekords gekontroleer sal word.

Aangesien siektetoestande die resultate van die studie mag beïnvloed, is dit vir ons belangrik om te weet of u siek was tydens die studie en of u enige medikasie gebruik het.

Indien u siek was tydens die studie, teken asseblief vir ons die datum aan, asook die medisyne wat u gebruik het vir die siektetoestand.

Lees asseblief die riglyne vir die neerskryf van die inligting en indien u enige probleme het, kan u Petro Wolmarans by 9320311 x 268 of Sulene Loots by 9320311 x 364 skakel.

Nogmaals baie dankie vir u deelname.

ALGEMENE INLIGTING

1. BLOEDTREK

In u dagboek (bladsye 7-10) sal u die datums vir u afsprake vind. Die bloed moet vastend getrek word, met ander woorde u mag vir 12 uur voordat die bloed getrek word, niks eet of drink nie. U mag wel water drink. Ons verkies ook dat u vir 24 uur voordat die bloed getrek word geen alkoholiese drank gebruik nie.

2. SIEKTETOESTANDE

Indien u siek was, dui asseblief aan op watter datum u siek was, hoe lank dit geduur het en wat u makeer het. Alle siektetoestande soos verkoue, griep, diarree, koors, enige infeksie ens. moet aangeteken word in u dagboek op bladsy 4.

3. MEDIKASIE

Indien u medisyne gebruik het vir die siektetoestand, gee asseblief die naam van die middel asook die hoeveelheid wat u gebruik het. Gee asseblief ook die naam van die inspuitings wat u gehad het, indien enige. Vra asseblief die naam daarvan vir u geneesheer. Sou u geneesheer besluit om die medisyne wat u gewoonlik gebruik te verander, teken dit asseblief ook in u dagboek aan. Gee die naam en die hoeveelheid. Moenie vergeet om ook enige vitamienpille wat u drink neer te skryf nie. *Dit is egter wenslik dat u geen vitamiensupplemente (pille of poeiers) gebruik tydens die studie nie.*

U MOET ASSEBLIEF NIE ENIGE VAN DIE VOLGENDE MIDDELS GEBRUIK TYDENS DIE STUDIE NIE:
MEDIKAMENTE WAT ASPIRIEN (ASETIEL-SALISIELSUUR) BEVAT OF ENIGE ANTI-INFLAMMATORIESE MIDDELS NIE, bv

Aspirien, Butazolidin, Tandril, Ponstan, Arlef, Voltaren, Fennopron, Naprosyn, Cinopal, Froben, Brufen, Indocid, Clinoril, Feldene, Panamor
antistolmiddels bv. Warferien, Heparien, Coumadin.

Sou u geneesheer enige van bogenoemde middels aan u voorskryf moet u asseblief een van die volgende persone kontak:

Dr JA Kriek 9320311 x 269 of mej P Wolmarans 9320311 x 268 of mej S Loots 9320311 x 364.

U mag die volgende gebruik indien nodig:

Calpol, Betapain, Beserol, Antipyn, Antiflu, Colcaps, Panado, Syndol, Sedinol, Sinutab, Stopayne, Dolorol.

4. DIEETREKORD

Volledige instruksies oor die hou van die dieetrekord sal mondelings en skriftelik aan u gegee word.

Dieet gedurende die studie;

(a) Eerste drie weke: u volg u normale dieet.

(b) Vierde tot die negende week: U volg vir ses weke die spesiale dieet wat vir u voorgeskryf word.

(c) Tiende tot die 15de week: U volg weer u normale dieet.

(d) Uitwasperiode - d.w.s. totdat die tweede deel van die studie begin volg u u normale dieet.

5. UITREIKING VAN VLEIS-, VIS- OF HOENDERPORSIES

Die datums waarop die voedselporsies aan u uitgereik sal word verskyn op bladsy 9.

6. VIR DIE DAMES

(a) **Voorbehoedmiddels**

Indien u voorbehoedmiddels gebruik, teken dit asseblief aan in u dagboek en gee die naam.

(b) **Menstruasiesiklus**

Teken asseblief tydens die studie die datum van die eerste en laaste dag van u menstruasie aan in u dagboek.

D A G B O E K

Datum		Siektetoestand	Medikasie voorgeskryf	Medikasie: Bestaande voorskrif verander
Van	Tot			

D A G B O E K

Datum		Siektetoestand	Medikasie voorgeskryf	Medikasie: Bestaande voor-skrif verander
Van	Tot			

D A G B O E K

Datum		Siektetoestand	Medikasie voorgeskryf	Medikasie: Bestaande voor-skrif verander
Van	Tot			

Groep 2

▲ Bloedtrek en Bloeddruk

◇ Lesing vir 7-dae rekord

□ 7-dae rekord begin

● Kontrolering van 7 dae rekord na 2 dae

◆ Kontrolering van 7 dae rekord na 7 dae

△ Lesing vir Eksperimentele dieet

○ Uitreiking van vleisporsies:

Elke Maandagmiddag na 16:00 vanaf 10 September 1990

1990 Jaarbeplanner

	JULIE	AUGUSTUS	SEPTEMBER	OKTOBER	NOVEMBER	DESEMBER
Maan				1 ○		
Dins				2		
Woens		1		3		
Don		2		4	1	
Vry		3		5	2	
Sat		4	1	6	3	1
Son	1	5	2	7	4	2
Maan	2	6	3 ◇	8 ○	5	3
Dins	3	7	4 ▲ □	9	6	4 ▲
Woens	4	8	5	10	7	5
Don	5	9	6 ●	11	8	6
Vry	6	10	7	12	9	7
Sat	7	11	8	13	10	8
Son	8	12	9	14	11	9
Maan	9	13	10 △ ○	15 ○	12	10
Dins	10	14	11 ▲ ◆	16 □	13	11
Woens	11	15	12	17	14	12
Don	12	16	13	18 ●	15	13
Vry	13	17	14	19	16	14
Sat	14	18	15	20	17	15
Son	15	19	16	21	18	16
Maan	16	20	17 ◇ ○	22	19	17
Dins	17	21 ▲	18 □	23 ▲ ◆	20	18
Woens	18	22	19	24	21	19
Don	19	23	20 ●	25	22	20
Vry	20	24	21	26	23	21
Sat	21	25	22	27	24	22
Son	22	26	23	28	25	23
Maan	23	27	24 ○	29	26	24
Dins	24	28	25 ▲ ◆	30	27	25
Woens	25	29	26	31	28	26
Don	26	30	27		29	27
Vry	27	31	28		30	28
Sat	28		29			29

7. **BELANGRIKE DATUMS EN PLEK VAN AFSPRAKE**

Normale dieet ..... tot .....

Eksperimentele dieet ..... tot .....

Normale dieet ..... tot .....

BASISLYN: (3 weke)

(a) Bloedtrek:

DATUM	TYD	PLEK

(b) Lesing: (7 dae dieetrekord)

DATUM	TYD	PLEK

(c) Bloedtrek

DATUM	TYD	PLEK

(d) Begin van 7 dae dieetrekord

DATUM	TYD	PLEK

(e) Kontrolering van 7 dae dieetrekord na 2 dae

DATUM	TYD	PLEK

(f) Lesing vir Eksperimentele dieet (oggend)

DATUM	TYD	PLEK

(g) Vleisuitreiking (middag):

DATUM	TYD	PLEK

(h) Kontrolering van 7 dae dieetrekord na 7 dae

DATUM	TYD	PLEK

(f) Bloedtrek

DATUM	TYD	PLEK

ADDENDUM E

MEDIESE NAVORSINGSRAAD

Navorsingstudie

EETPATROON EN RUILLYSTE

INHOUDSOPGAWE

DIEETVOORSKRIF VIR DIE EKSPERIMENTELE PERIODE

Gedurende die volgende ses weke word 'n spesiale dieet vir u voorgeskryf. Die doel met hierdie dieet is om die bloedvetvlakke te verlaag ten einde die risiko vir koronêre hartsiekte (hartaanvalle) te verminder.

U huidige gewig en vlak van aktiwiteit word in aanmerking geneem by die beplanning van die dieet. Almal kry dus nie dieselfde dieet nie, en dis belangrik dat u u eie dieet volg! Kontak ons asseblief indien u gewig optel of gewig verloor op die dieet sodat die nodige aanpassings gemaak kan word.

Die dieet bestaan uit 'n DAAGLIKSE VOEDSELRANTSOEN (bladsy 2) en 'n RUILLYS (bladsy 3). Die daaglikse voedselrantsoen dui aan hoeveel voedsel u daagliks uit elk van die ses voedsel groepe moet eet. U word aangeraai om nie meer maar ook nie minder te eet as wat voorgeskryf is nie. In die Ruillys word voedsel in ses verskillende voedselgroepe ingedeel naamlik die:

Melk of vervangergroep

Vleis of vervangergroep

Brood of vervangergroep

Olie of vervangergroep

Vrugtegroep

Groentegroep

Voedsel wat saamgegroepeer is in 'n groep bevat vir 'n spesifieke hoeveelheid voedsel ongeveer dieselfde hoeveelheid koolhidrate (stysel), proteïen, vet en energie (kilojoules). Voedsel wat in dieselfde groep gegroepeer is, mag dus vir mekaar uigeruil word.

Daaglikse voedsel rantsoen

Kies daagliks 'n verskeidenheid van voedsel uit elk van die ses voedselgroepe. U word aangeraai om niks meer maar ook niks minder te eet as die hoeveelheid voedsel wat vir u vooorgeskryf is nie. Indien u gewig optel of gewig verloor kontak ons asseblief.

Eet elke dag

.....Melkruile of vervanger

.....Vleisruile of vervanger

.....Broodruile of vervanger

.....Olieruile of vervanger

.....Vrugteruile

.....Groenteruile

Verdeel die voedsel wat toegelaat word per dag tussen die verskillende maaltye van die dag volgens u eie behoefte en eetpatroon. U mag ook van die voedsel toegelaat per dag tussen maaltye eet.

RUILLYS

Melk en vervangergroep

Kies daagliks .....ruile uit hierdie groep.

Voedsel toegelaat	Hoeveelheid gelyk aan een ruil
Laevet melk (2%)	250 ml
Laevet karringmelk	250 ml
Laevet jogurt	250 ml

Praktiese wenke

* Gebruik 250 ml vars afgeroomde melk of 60 ml (25 g) afgeroomde poeiermelk plus een olieruil in plaas van 250 ml laevet melk (2%).

* Gebruik laevet melk (2%) in die voorbereiding van voedsel en in gebak.

* Maak u eie vars vrugtejogurt deur onversoete laevet jogurt met 'n vrugteruil, byvoorbeeld 200 g vars aarbeie te meng.

* Eet vetvry of laevet jogurt in plaas van roomys saam met vrugteslaai.

* Vervang 250 ml laevet melk (2%) plus 2 vrugteruile met 175 ml laevet vrugtejogurt.

Voedsel om te vermy.

Volmelk, koffieverromers, mengsels van nie-suiwelverromers en melkpoeier ("blends"), roomys, drinksjokolade en soortgelyke dranke, melkskommels, volvet jogurt.

Vleis of vervangergroep

Kies daagliks .....ruile uit hierdie groep.

Voedsel toegelaat (gaar gewig)	Hoeveelheid gelyk aan een ruil
Maer beesvleis	30 g (50 mm x 30 mm x 20 mm)
Maer gemaalde beesvleis	30 g (2 gelykvol eetlepels)
Maer skaap- of varkvleis	30 g
Eier	1 (slegs 2 eiergele per week)
Vetvry of laevet maaskaas	50 g (60 ml)

Die vleis wat verskaf word, is die aantal vleisruile wat u per week moet eet. U word vriendelik versoek om nie meer maar ook nie minder vleis te eet as wat verskaf word nie. Die sukses van die studie hang daarvan af en ons maak staat op u goeie samewerking.

U word versoek om nie meer as twee eiergele per week te eet nie. Die twee eiers is gelyk aan twee vleisruile dws 2 x 30 g vleis. Eet slegs een eier per keer en wel saam met die maer maalvleis en die laevet wors (daar is een porsie laevet maalvleis en een porsie laevet wors in u vleispakket vir die week). U moet asseblief geen harde kaas bv Cheddar, soetmelk, Feta, Camembert ens of smeerkaas, gedurende die eksperimentele periode eet nie. U mag wel maaskaas eet.

Praktiese wenke

* Eet slegs maer vleissnitte
* Sny die vet van die vleis af voor gaarmaak.
* Rooster, bak of kook die vleis. Moenie in vet braai nie.
* Plaas vleissous in die koelkas. Laat die vet hard word en verwyder dit. Gebruik die vetvrye vleissous.
* Kies resepte wat min of geen eiers bevat.
* Gebruik vetvry of laevet maaskaas in plaas van harde kaassoorte in soutgeregte.

Voedsel om te vermy

Hoender; alle vissoorte insluitende kaviaar, viskuit, garnale, steurgarnale en kreef; vetterige vleis bv wors, polonie, spek, murg, weense worsies; verdikte en vetterige vleissous en sop; harsings, lewer, niertjies en ander organe; gebraaide vleisgeregte, behalwe met olie van daaglikse rantsoen, vleis bedek met krummels of beslag; harde kaas en smeerkaas.

Vleis of vervangergroep

Kies daagliks.....ruile uit hierdie groep

Voedsel toegelaat	Hoeveelheid gelyk aan een ruil
Hoender (sonder vel)	30 g
Tuna	30 g
Vetryke vis bv snoek	30 g
Witvis bv stokvis	40 g
Sardyne in tamatiesous	40 g
Eier	1 (slegs 2 eiergele per week)
Vetvry of laevet maaskaas	50 g (60 ml)

Die vis en hoender wat verskaf word, is die aantal vleisruile wat u per week moet eet. U word vriendelik versoek om nie meer maar ook nie minder vis en hoender te eet as wat verskaf word nie. Die sukses van die studie hang daarvan af en ons maak staat op u goeie samewerking.

U word versoek om nie meer as twee eiergele per week te eet nie. Die twee eiers is gelyk aan twee vleisruile dws 2 x 30 g vis of hoender. Eet slegs een eier per keer en wel saam met die hoenderborsies (daar is twee porsies hoenderborsies per week in u vleispakket). U moet asseblief geen harde kaas bv Cheddar, soetmelk, Feta, Camembert ens of smeerkaas, gedurende die eksperimentele periode eet nie. U mag wel maaskaas eet.

Praktiese wenke

* Kies resepte wat min of geen eiers bevat.

* Gebruik afgeroomde melk uit die rantsoen en maak 'n geurige witsous by die vis. Gee die witsous met sout, peper en ander speserye in plaas van met kaas.

* Gebruik vetvry of laevet maaskaas in plaas van harde kaassoorte in soutgeregte.

Voedsel om te vermy

Skaap- bees- en varkvleis; ingemaakte vleis; vetterige vleis bv wors, polonie, spek, murg, weense worsies; verdikte en vetterige vleissous en sop; harsings, lewer, niertjies en ander organe; kaviaar, viskuit, garnale en steurgarnale. Gebraaide vis/hoendergeregte, behalwe met olie van daaglikse rantsoen; vis of hoender bedek met krummels of beslag; harde kaas en smeerkaas.

Brood of vervangergroep

Kies daagliks.....ruile uit hierdie groep

Voedsel toegelaat	Hoeveelheid gelyk aan een ruil
Brood wit, bruin, volkoring	1 sny (93 mm x 93 mmx 10 mm) (30 g)
Broodrolletjie	1 klein (100 mm x 40 mm deursnee) (30 g)
Hamburger broodrolletjie	1/2 (100 mm x 40 mm deursnee) (30 g)
Kraakbeskuitjies	3-4 (18-20 g)
Hawermoutpap	125 ml (125 g)
Mieliepap	130 ml (125 g)
Kitsontbytgraan	125 ml (125 g)
All Bran	150 ml (30 g)
Weetbix	1 blokkie (25 g)
Gekookte aartappel	1 medium (60 mm x 50 mm) (90 g)
Kapokaartappels	125 ml (120 g)
Patats	60 ml (70 g)
Gaar droëbone	100 ml (80 g)
Gaar droë-erte	100 ml (80 g)
Gaar lensies	100 ml (70 g)
Rys	125 ml (65 g)
Stampmielies	125 ml (115 g)
Stampkoring	150 ml (100 g)
Suikermielies	125 ml (110 g)
Groenmielie	1 medium (125 mm x 45 mm) (130 g)
Gaar pasta	125 ml (80 g)
Springmielies	300 ml (20 g)

Praktiese wenke

* Kies volgrane in plaas van verfynde grane
* Maak witsous van laevet (2%) melk en meel maar sonder botter, olie of margarien.
* Ruil een sny brood vir 250 ml groentesop sonder vleis.
* Indien u poli-onversadigde margarien of olie in gebak gebruik, mag u af en toe as volg ruil:

Ruil vir 1 sny brood en 2,5 ml olie:

2 (2 x 10 g) eenvoudige droëkoekies

Ruil vir 1 sny brood en 5 ml olie:

1 (30 g) botterbroodjie (60 mm deursnee x 25 mm)

Ruil vir 1 sny brood en 10 ml olie:

1 (30 g) pakkie harde aartappelskyfies

Ruil vir 1½ snye brood en 5 ml olie:

1 (30 g) volkoringbeskuit (80mm x 28 mm x 30 mm)

Ruil vir 1½ snye brood en 10 ml olie:

1 (70 g) pannekoek 170 mm deursnee OF 10 (60 g) vars aartappelskyfies (110 mm 10 mm x 8mm)

Ruil vir 2 snye brood en 5 ml olie:

1 (50 g) stuk eenvoudige koek (75 mm x 75 mm x 20 mpm)

Voedsel om te vermy

Witsouse gemaak van volmelk, koek, tert,pasteie, kommersiële droëkoekies, ryk gebakte nageregte en pizzas

Olie of vervangergroep

Kies daagliks.....ruile uit hierdie groep

Voedsel toegelaat	Hoeveelheid gelyk aan een ruil
Poli-onversadigde margarien (50% poli-onversadig) bv Floro	5 ml
Poli-onversadigde margarien (medium vet) bv Florolite	7 ml
Lae kilojoule slaaisous bv Trim	25 ml
Okkerneute	2
Amandels	4

Praktiese wenke

* Margarien (in bakkies met 50% of meer poli-onversadigde vette) en olie - gebruik slegs die hoeveelheid wat voorgeskryf is omdat dit die totale vetinhoud van die dieet verhoog. Smeer die brood DUN.

* Vermy sover as moontlik die gebruik van botter, margarien, olie of ander vette in die voorbereiding van voedsel.

* Af en toe kan een van die volgende geruil word vir 5 ml olie:

  Avokado = 25 g (100 mm x 25 mm)

  Olywe = 8

  Olyfolie = 5 ml

Voedsel om te vermy

Gebraaide voedsels, behalwe met olie van daaglikse rantsoen; harde margarien, botter, room (vars of ingemaak), nagemaakte room, tuisgemaakte mayonnaise (met eiergeel), gekoopte mayonnaise, neute behalwe okkerneute en amandels (sien plaasvervangers vir olie), klapper, klapperolie en palmolie).

Vrugtegroep

Kies daagliks.....ruile uit hierdie groep

Voedsel toegelaat	Hoeveelheid gelyk aan een ruil
Vars vrugte	
Aarbeie *	300 ml (200 g)
Appel	1 (60 mm deursnee) 100 g
Appeliefies	200 ml (140 g)
Appelmoes (onversoet)	125 ml (140 g)
Appelkose - vars	4 (40 mm deursnee) 140 g
Druiwe (klein trossie)	15 korrels (90 g)
Granadillas	2 (48 mm x 50 mm) (60 g)
Kersies - vars -groot	14 (90 g)
Kiwi (58 mm x 43 mm)	1 (80 g)
Koejawel* - groot	1 (65 mm x 55 mm deursnee) (130 g)
Kweper (medium)	1/2 (75 mm x 73 mm) (125 g)
Lemoen *(klein) geskil	1 (60 mm deursnee) (150 g)
Lietjies* (klein)	12 (25 mm x 20 mm deursnee) (100 g)
Lukwarte	8 (40 mm x 35 mm) (120 g)
Nartjie * - geskil	2 (52 mm x 39 mm) (150 g)
Papajablokkies*	300 ml (175 g)
Papajaskyf*	1 skyf 175 g
Peer - vars - klein	1 (60 mm x 52 mm) (100 g)
Perske - vars- medium	1 (65 mm x 60 mm) (150 g)
Piesang - klein	1 (110 mm x 25 mm) (70 g)
Pomelo* - klein	1 (88 mm deursnee) (180 g)
Pomelosegmente *	180 ml (180 g)
Pruime - medium	2 (45 mm x 40 mm) (100 g)
Pynappel	3 (85 mm x 10 mm) (120 g)
Pynappelblokkies	200 ml (120 g)
Spanspek* - groen skil	1/3 van 110 mm deursnee (200 g)
Spanspek* - skurwe skil	1/3 van 110 mm deursnee (200 g)
Spanspekblokkies*	300 ml (200 g g)
Turksvy - medium	2 (65 mm x 48 mm) (150 g)
Veselperske* - rond	1 (70 mm deursnee) (100 g)
Veselperske* - lank	1 (100 mm x 45 mm) (100 g)
Vy, vars	2 (45 mm x 44 mm) (80 g)
Waatlemoenskyf	1 (330 mm x 60 mm) (200 g)
Waatlemoen - kroonstukke	2 (70 mm x 25 mm) (200 g)

Vrugtegroep (vervolg)

Voedsel toegelaat	Hoeveelheid gelyk aan een ruil
Droë vrugte	
Appels	5 ringe (25 g)
Appelkose	8 halwes (30 g)
Dadels - medium	4 (20 g)
Pere	2 halwes (24 g)
Perskes - medium	2 halwes (24 g)
Pruimedante	3 klein of 2 groot (24 g)
Rosyntjies	1 eetlepel (20 g)
Vy	1 (50 mm deursnee x 10 mm) (20 g)
Vrugtesap	
Appelsap	125 ml
Druiwesap	100 ml
Koejawelsap -onversoet	100 ml
Lemoensap*	125 ml
Pomelosap*	150 ml
Pruimedantesap-onversoet	80 ml
Pynappelsap-onversoet	100 ml

Die gewigte is vir die geskilde vrug waar van toepassing.

Praktiese wenke.

* Eet ten minste 1 ruil van hierdie vitamien C ryk vrugte per dag

- Eet vars vrugte in plaas van nagereg

- 'n Heel vrug is meer vullend as vrugtesap.

Voedsel om te vermy

Ingemaakte vrugte, behalwe in natuurlike sap (sien vrugtelys); versuikerde vrugte.

Groentegroep

Kies .....ruile groente uit hierdie groep.

125 ml (½ koppie) groente is gelyk aan 1 ruil.

Andyvie	Murgpampoen
Aspersie	Pietersielie*
Beet	Preie
Blaarslaai	Radyse
Blomkool	Rape
Boerpampoen*	Rubarber
Botterskorsie*	Sampioene
Brokkolie*	Seldery
Bronkors*	Skorsie
Eiervrug	Soetrissie
Geelwortels*	Spinasie*
Groenboontjies	Spruikool
Groenertjies	Suurkool
Hubbardpampoen*	Tamaties
Jongui (Spring onion)	Tamatiesap - 180 ml
Komkommer	Uie
Kool	

Praktiese wenke

* Eet een ruil karoteenryke groente per dag

- Eet daagliks een of meer groentes rou
- Eet 'n verskeidenheid van groente

Voedsel om te vermy

Groente met bygevoegde suiker; ryk kaassouse by groente; botter en harde margarien en olie by groente (tensy as deel van die daaglikse rantsoen).

Suiker en ander soetgoed

Suiker en soetgoed het 'n hoë energie-inhoud en verskaf nie vitamiene en minerale nie.

Beperk soetgoed streng indien u oorgewig is.

Indien u suiker gebruik moet 15 ml suiker geruil word vir een sny (30 g) brood (een broodruil).

U mag daagliks hoogstens .....ml suiker gebruik

Vervangers vir suiker

Ruil 5 ml suiker vir:

5 ml konfyt OF 5 ml heuning OF 5 ml stroop OF 3 vrugte gommetjies

Ruil 2 x 5 ml suiker vir:

1 marshmallow OF 60 ml jellie OF 3 jellie boontjies

Ruil 3 x 5 ml suiker vir:

2 "jelly babies" (3 g elk) OF 4 peppermente (3 g elk) OF 2 harde suiglekkers (7 g elk)

Ruil 6 x 5 ml suiker vir:

250 ml aangemaakte koeldrank

Ruil 9 x 5 ml suiker vir:

340 ml (1 blikkie) gaskoeldrank.

Voedsel om te beperk soos aangedui

Lekkers; konfyt; stroop; heuning; suiker (bruin en wit); koeldrank; gemmerbier; limonade ens. (beperk soos aangedui onder wenke).

Voedsel om te vermy

Sjokolade; toffies; fudge.

Alkoholiese drank

Indien u alkohol gebruik word twee alkoholiese drankies per dag toegelaat. Ruil asseblief as volg indien u alkohol wil gebruik:

340 ml gewone bier vir:

1½ broodruil

120 ml wyn, wit - medium en soet vir:

1½ broodruil

120 ml droë wit, rooi, rosé wyn vir:

1 broodruil

60 ml port, soet sjerrie, muskadel vir:

1 broodruil

30 ml likeur vir:

1 broodruil

25 ml gin, brandewyn, whisky vir:

3/4 broodruil

60 ml sjerrie - droog/medium, vermoet - droog vir:

3/4 broodruil

ADDENDUM F

'N PAAR IDEES VIR KOSMAAK MET ROOIVLEIS

(WEEK I)

Gaarmaak van vleis:

- Sny alle sigbare vet af.

- Gebruik gerus sout, peper, Aromat, Worcestersous, sojasous, kruie, speserye en asyn vir smaak.

- Ruil 25 mL (g) tamatiesous vir ½ suikerruil.
  5 mL (g) blatjang vir ½ suikerruil.

- Droë wyn (as broodruil) kan ook in marinade gebruik word.

- Die vleis kan verder gebraai word in 'n pan (gebruik van die olieruile).

- Kan gerooster word oor die kole (geur met bg bestanddele, of maak selfs marinade van bestanddele toegelaat).

- Lasanga kan gemaak word met maalvleis:
  pasta afkomstig van broodruil,
  witsous, gebruik van 2% melkrantsoen
  gebruik van olieruile
  en meel: 50 mL koekmeel = 1 broodruil.

Toebroodjie idees!

- Marmite kan vrylik gebruik word en Fray Bento's.
- "Sandwich spread" ruil as mayonnaise.
- Tamatie, komkommer, kropslaai, ens. as groenteruile op brood.
- Piesang, as vrugteruil, op brood.
- Maaskaas kan 2x in die week geëet word.

Dranke:

Dieetkoeldranke en suikerpilletjies - vrylik!

NOG 'N PAAR KOSMAAKIDEES!

(WEEK II)

"In Shape" roomys kan gebruik word in die plek van 2% melk, bv 100 mL roomys = 100 mL 2% melk.

Vla (50 mL/50 g) = 50 mL 2% melk
1 suikerruil

1 Vrug = 125 g (150 mL) vrugteslaai

Vrugteslaai saam met 175 mL Bulgaarse Joghurt maak 'n heerlike nagereg! Voeg suiker (suikerpilletjies) by indien verkies.

Nog idees met vleis!

Beesvleis sosaties kan gemaak word van die beesvleisstukkies. Saam met die beesvleisstukkies kan uie, sampioene, pynappelstukkies en droë vrugte ingeryg word.

'n Lekker marinade resep:

20 mL Worcestersous/Sojasous
30 mL Asyn/Suurlemoensap
30 mL Sonneblomolie (6 olieruile)
1 ui gekap (1/4 groenteruil)

Dié resep is vir 4 mense. Die olieruile wat prysgegee moet word, is 1½ en die groenteruil is weglaatbaar.

Toebroodjie idees!

- Marmite kan vrylik gebruik word en Fray Bento's.
- "Sandwich spread" ruil as mayonnaise.
- Tamatie, komkommer, kropslaai, ens. as groenteruile op brood.
- Piesang, as vrugteruil, op brood.
- Maaskaas kan 2x in die week geëet word.

Dranke:

Dieetkoeldranke en suikerpilletjies - vrylik!

'N PAAR IDEES VIR KOSMAAK MET HOENDER EN VIS

(WEEK I)

- Gebruik gerus sout, peper, Aromat, Worcestersous, sojasous, kruie, speserye en asyn vir smaak.

- Ruil 25 mL (g) tamatiesous vir ½ suikerruil
  5 mL (g) blatjang vir ½ suikerruil

- Droë wyn (as broodruil) kan ook in die voorbereiding van vis/hoender gebruik word.

- Die hoender kan gerooster word oor die kole (geur met bogenoemde bestanddele of maak selfs marinade van bestanddele toegelaat).

- Die hoender kan in die oond gebak word - bedruip af en toe met die ruile toegelaat.

- Sampioene en uie kan gesoteer word in die pan, en die ontbeende hoender kan daarby gevoeg word.

- Hoender-lasanga kan gemaak word van ontbeende hoender, pasta (broodruil) en witsous - 2% melk) rantsoen
  - margarien)
  en koekmeelblom - 50 mL = 1 broodruil.

- Vis of hoender kan gerol word in melk (2%), daarna in koekmeelblom of krummels van graanvlokkies (broodruile), en dan

in 'n pan saam met vetruile gebak word.

Toebroodjie idees!

- Marmite & Fray Bentos kan vrylik gebruik word.
- "Sandwich spread" ruil as mayonnaise.
- Tamatie, komkommer, kropslaai, ens as groenteruile op brood.
- Piesang as vrugteruil op brood.
- Maaskaas kan 2x 'n week geëet word.

Dranke

Dieetkoeldranke en suikerpilletjies vrylik!

NOG 'N PAAR KOSMAAKIDEES MET HOENDER EN VIS

(WEEK II)

Nageregte

- "In Shape" roomys kan gebruik word in die plek van 2% melk, bv 100 mL roomys = 100 mL 2% melk.

- Vla (50 mL/50 g) = 50 mL 2% melk
  = 1 suikerruil

- 1 Vrug = 125 g (150 mL) vrugteslaai
  Vrugteslaai saam met 175 mL Bulgaarse Joghurt maak 'n heerlike lae vet nagereg!

Nog idees vir die hoender-mense!

- Hoender sosaties kan gemaak word van die borsie-gedeelte van die hoender. Saam met die hoender kan uie, sampioene, pynappelstukkies en droë vrugte ingeryg word.

Marinade: (4 porsies; 1 porsie = 1 broodruil)

250 mL witwyn
1 huisie knoffel fyngedruk
12,5 mL sojasous
5 mL mosterdpoeier
sout en peper na smaak
5 mL fyngedrukte roosmaryn
12,5 mL suurlemoensap
5 mL suiker
10 mL mielieblom met 'n bietjie water gemeng.

Meng bestanddele vir marinade. Giet oor sosaties en marineer oornag in yskas. Verwyder van marinade en rooster 10 cm vanaf hittebron vir sowat 20 min. Bedruip gedurig met orige marinade. Die marinade wat verder mag oorbly, kan verhit word en verdik word met mielieblom en as sous oor die sosaties bedien word.

- Nog 'n idee met hoender is om dit ook weer in blokkies te sny en te roerbraai saam met uie, sampioene, knoffel, groenrissie, wortels, murgpampoentjies en gekookte noedels. Die wortels en murgpampoentjies moet ook effens gekook word, voordat dit by die roerbraai gevoeg word.

Hier is 'n lekker sous waarin die hoender en groente geroerbraai kan word:

180 mL water]
½ hoendergeurblokkie] los op
10 mL sojasous
10 mL suurlemoensap
25 mL sjerrie
10 mL mielieblom

Meng bestanddele en voeg by roerbraai. Die resep is vir 6 porsies en geen ruile hoef prysgegee te word nie!

'n "Geblikte" vis idee:

Al wat vir die resep benodig word, is:

Tuna of Pilchards (wat voorsien word)
1 k gekookte noedels (2 broodruile)
1 ui, geskil (1/4 groenteruil)
Geurmiddels - net wat u verkies!
1 k witsous : 25 mL margarien (5 olieruile)
: 25 mL koekmeelblom (½ broodruil)
: 200 mL 2% melk (vir elke 250 mL melk wat ekstra gebruik word, moet 1 broodruil + 1 vetruil prysgegee word).

Metode:

Soteer die uie. Voeg die tuna of pilchards by en verwarm effens. Voeg die noedels en witsous by. Meng goed en bak vir 10-15 min by 180°C.

As laaste gedagte:

Plaas gegeurde hoender saam met 250 mL appelkoossap (Liquid Fruit) in oondskottel en bak vir een uur by 180°C. Die appelkoossap = 2½ vrugteruile.

Dié resep is vir dié mense wat radeloos is om hul vrugteruile op te kry!

ADDENDUM G

MEDIESE NAVORSINGSRAAD
NAVORSINGSINSTITUUT VIR VOEDINGSIEKTES

NAVORSINGSPROJEK: DIEETBEHANDELING VIR VERHOOGDE BLOEDCHOLESTEROL

Vraelys in verband met die eksperimentele dieet

Gedurende die eksperimentele periodes van die navorsingsprojek moes u 'n spesiale voorgeskrewe eetpatroon volg. Belangrike inligting in verband met die navolging en aanvaarbaarheid van die eetpatroon word verlang.

Voltooi asseblief die onderstaande vraelys.
Merk die toepaslike blokkie met 'n X.

Kantoorgebruik

Kodenommer ☐☐☐ 3

Vraag 1
Watter eetpatroon moes u volg gedurende:

a) fase 1 (1990) van die eksperimentele periode

(1) net rooivleis ☐

(2) net hoender en vis ☐

b) fase II (1991) van die eksperimentele periode

(1) net rooivleis ☐

(2) net hoender en vis ☐ 5

Kantoorgebruik

Vraag 2
Indien u vir uself 'n punt uit 10 moet gee (waar 10 uit 10 baie goed is), hoe goed sou u sê het u die voorgeskrewe eetpatroon gevolg?

a) Eksperimentele dieet met rooivleis

1	2	3	4	5	6	7	8	9	10

b) Eksperimentele dieet met hoender en vis

1	2	3	4	5	6	7	8	9	10

7

Vraag 3
Wat was die moeilikste dieetaanpassing(s) wat u moes maak gedurende die eksperimentele periode. (Noem asb)

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.....

.....

Vraag 4

4.1 Het u soms <u>rooivleis</u> geëet gedurende die eksperimentele periode waartydens u hoender en vis moes eet?

JA ☐ NEE ☐

Indien JA, hoeveel keer ☐

4.2 Het u soms <u>hoender en vis</u> geëet gedurende die eksperimentele periode waartydens u rooivleis moes eet?

JA ☐ NEE ☐

Indien JA, hoeveel keer hoender ☐

hoeveel keer vis ☐

15

Kantoorgebruik

Vraag 5

U het 'n voorgeskrewe porsie rooivleis OF hoender en vis ontvang. Dui asseblief aan of die porsie te veel, te min of net reg was.

Rooivleis:

- Te veel ☐
- Te min ☐
- Net reg ☐

Hoender:

- Te veel ☐
- Te min ☐
- Net reg ☐

Vis:

- Te veel ☐
- Te min ☐
- Net reg ☐

☐ 18

Kantoorgebruik

Vraag 6
Hoe goed het u gehou by die hoeveelhede voedsel wat vir u voorgeskryf is?
(Merk die toepaslike blokkie met 'n X)

Fase I (1990)

	1 100%	2 75-100%	3 50-75%	4 <50%
Melkruile				
Vleisruile				
Broodruile				
Olieruile				
Groenteruile				
Vrugteruile				

Fase 2 (1991)

	1 100%	2 75-100%	3 50-75%	4 <50%
Melkruile				
Vleisruile				
Broodruile				
Olieruile				
Groenteruile				
Vrugteruile				

Vraag 7:
Hoeveel eiers (net so of in geregte) het u gemiddeld per week geëet gedurende die eksperimentele periode?

Aantal eiers per week terwyl op rooivleis ☐ ☐☐

Aantal eiers per week terwyl op hoender ☐ ☐☐

34

Kantoorgebruik

Vraag 8:
Hoeveel keer het u harde kaassoorte (bv cheddar, soetmelk, feta ens) net so geëet of in geregte, gedurende die ses weke eksperimentele periode?

Fase 1 (1990)

aantal kere per dag =

aantal kere per week =

aantal kere in ses weke =

Fase 2 (1991)

aantal kere per dag =

aantal kere per week =

aantal kere in ses weke =

Vraag 9
Het u gereeld (meer as drie keer per week) die voorskrif in verband met die daaglikse gebruik van alkoholiese drank oorskry?

JA NEE

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Vraag 10:
Normaalweg, hoeveel keer per week sou u graag die volgende wou eet?

(1) rooivleis

(2) hoender

(3) vis

Kantoorgebruik

Vraag 11:
Sien u kans om die eksperimentele dieet as 'n nuwe eetpatroon te aanvaar, mits u toegelaat word om maer rooivleis, hoender sonder vel en vis te eet?

JA ☐ NEE ☐ ☐ 46

Indien NEE waarom nie (Gee redes asseblief)

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Vraag 12:
Enige ander opmerkings (bv. het sekere van u eetgewoontes permanent verander agv die dieetvoorskrif)

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Baie dankie vir u vriendelike samewerking en u volgehoue belangstelling, ons waardeer dit baie.