THE EFFECT OF DIETARY FAT SOURCE ON CHOLESTEROL METABOLISM AND PLASMA PHOSPHOLIPID FATTY ACID PATTERNS IN HYPERLIPIDEMIC MEN

BY

DIANA S.H. CHARD

A Thesis

submitted to the Faculty of Graduate Studies

in Partial Fulfillment of the Requirements

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ABSTRACT

A metabolic study was undertaken to assess the effect of dietary oleic acid (OA), linoleic acid (LA) and linolenic acid (LNA) on plasma lipid metabolism in hyperlipidemic men. The study was comprised of two 48-day replicates; each was divided into a 6-day pre-experimental period, two 18-day experimental periods and a 6-day post-experimental period. The diets provided 51% of energy (3200 kcal/day) as carbohydrate, 13% as protein, and 36% as fat. Added fat (81% of total fat) was supplied by a mixture of fats during the pre- and post-experimental periods. Four sources of added fat, viz. (i) sunflower and olive (S/O), (ii) canola (CAN), (iii) soybean (SOY), and (iv) sunflower, olive and flax (S/O/F) oils which provided similar amounts of saturated fatty acids but different amounts of OA, LA and LNA, were used during the experimental periods. There were eight subjects in the first replicate and seven in the second (two subjects served in both replicates). Fasting (12-hour) blood samples were analyzed for plasma lipid and lipoprotein and for the fatty acid composition of plasma phosphatidylcholine, phosphatidylethanolamine, alkenylacyl ethanolamine phosphoglyceride, and cholesteryl esters (CE). A significant reduction ($p < 0.05$) in mean plasma total and LDL-cholesterol levels was observed in the CAN and SOY groups at the end of Experimental Period I. Similar but non-significant decreases were observed on the S/O and S/O/F diets. Dietary fat source

had no effect on HDL- and VLDL-cholesterol levels, while triglyceride levels were significantly lower on the S/O/F diet than on the other three diets. Diets containing high amounts of OA, LA and LNA were associated with significantly higher levels of OA, LA and LNA, respectively, in plasma phospholipids and CE. The SOY and S/O/F diets were associated with significantly lower levels of eicosatrienoic acid, while the CAN and S/O/F diets were associated with significantly higher levels of eicosapentaenoic acid in the plasma lipids. Diets had no effect on arachidonic, docosatetraenoic, docosapentaenoic, docosahexaenoic acids, or total n-6 and n-3 polyunsaturated fatty acids (PUFA). The results indicated that dietary OA, LA and probably LNA had similar cholesterol-lowering effects in hyperlipidemic subjects. Dietary fatty acids also had an influence on plasma lipid fatty acid metabolism. Dietary LA/LNA ratio appeared to influence n-3 PUFA metabolism, whereas the absolute amounts of dietary LA and LNA were more important in influencing n-6 PUFA metabolism in the hyperlipidemic individuals.

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LIST OF ABBREVIATIONS

Adenosine Diphosphate	ADP
Alkenylacyl Ethanolamine Phosphoglyceride	PPE
Arachidonic Acid	AA
Canola	CAN
Carbohydrate	CHO
Chloroform	$CHCl_3$
Cholesteryl Esters	CE
Coronary Heart Disease	CHD
Docosahexaenoic Acid	DHA
Docosapentaenoic Acid	DPA
Docosatetraenoic Acid	DTA
Eicosapentaenoic Acid	EPA
Eicosatrienoic Acid	ETA
Familial Hypercholesterolemia	FH
Gas Chromatography	GC
High Density Lipoprotein-Cholesterol	HDL-C
Least Significant Difference	LSD
Linoleic Acid	LA
α-Linolenic Acid	LNA
Low Density Lipoprotein-Cholesterol	LDL-C
Methanol	CH_3OH
Monounsaturated Fatty Acids	MUFA
Oleic Acid	OA
Palmitic Acid	PMA

ABBREVIATIONS (cont'd).

Pentadecaenoic Acid	15:0
Phosphatidylcholine	PC
Phosphatidylethanolamine	PE
Phospholipids	PL
Polyunsaturated Fatty Acids	PUFA
Prostacyclins	PGI
Saturated Fatty Acids	SFA
Sodium Methoxide	$NaOCH_3$
Soybean	SOY
Stearic Acid	STEA
Sunflower and Olive	S/O
Sunflower, Olive and Flax	S/O/F
Thin-Layer Chromatography	TLC
Thromboxane	TX
Total Cholesterol	TC
Triglyceride	TG
Very Low Density Lipoprotein-Cholesterol	VLDL-C

1. LITERATURE REVIEW

1.1 Introduction

Coronary heart disease (CHD) is the leading cause of mortality and morbidity in the industrialized nations. According to Statistics Canada (1988), CHD was the most frequent cause of death among men in the 45-64 age range, while it was the leading cause of death for both men and women aged 65 and over. CHD constitutes two major events, namely, atherosclerosis and thrombosis. Atherosclerosis starts with damage to the endothelium of an artery. Over a period of time, a thrombus eventually obstructs the artery, causing myocardial infarction, which may result in fatal arrhythmias (Dodson & Horton, 1987; Leaf & Weber, 1988; Ross, 1986).

Saturated fatty acids (SFA) have been consistently proven to be hypercholesterolemic, i.e. increase plasma cholesterol or more specifically low-density lipoprotein cholesterol (LDL-C) (Grundy & Denke, 1990; Hegsted et al., 1965; Keys et al, 1957). The hypocholesterolemic effect of oleic acid (OA) and linoleic acid (LA) has been clearly demonstrated as well (Baggio et al., 1988; Chan, 1990; Mattson & Grundy, 1985; McDonald et al., 1989; Mensink & Katan, 1987, 1989). However, the hypocholesterolemic effect of α-linolenic acid (LNA) requires further clarification. In addition, the mechanisms through which these fatty acids affect the cholesterol metabolism are unclear.

LA and LNA are of significance as well in their effects

on thrombosis. It has been proposed that LA and LNA can be desaturated and elongated to arachidonic acid (AA) and eicosapentaenoic acid (EPA), respectively. AA is the precursor of vasoconstrictive and proaggregatory thromboxane A_2 (TXA_2) while EPA is the precursor of a very weak platelet aggregator TXA_3. Both AA and EPA also synthesize vasodilative and antiaggregatory prostanoids, namely prostaglandin I_2 (PGI_2) and PGI_3, respectively (Herold & Kinsella, 1986; Leaf & Weber, 1988). Since dietary LA and LNA share the same enzyme systems in the desaturation and chain elongation pathways (Cook, 1985), the absolute and relative amounts of these fatty acids are anticipated to have an influence on the metabolism of n-6 and n-3 polyunsaturated fatty acids (PUFA). This, in turn, will have an effect on the prostanoid metabolism and thrombosis.

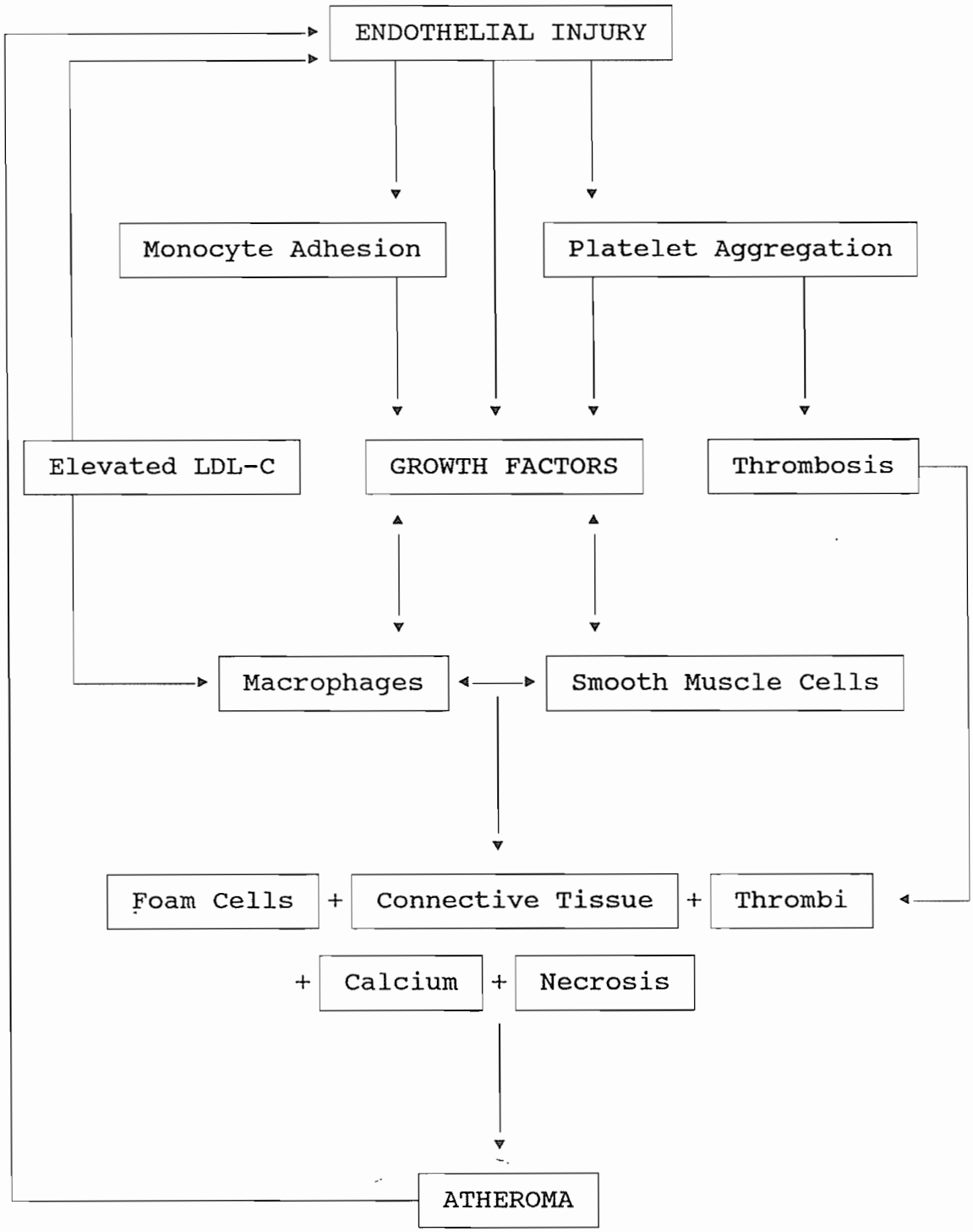
Due to the prevalence of CHD in industrialized nations such as Canada, the study of the effect of dietary fat source on plasma lipid metabolism should not only be given attention but should take precedence in research. Since more than half of all the adults in the industrialized world have plasma cholesterol greater than or equal to 4.7 mmol/l (Nestel, 1987), it becomes crucial to advise the entire community to modify their dietary habits, especially those with elevated cholesterol levels. Information generated from this research will be very useful for this purpose and the dietary treatment of hypercholesterolemia.

1.2 Pathogenesis of CHD

The pathogenesis of CHD has been described by several authors (Betteridge, 1987; Dodson & Horton, 1987; Leaf & Weber, 1988; Ross, 1986). An outline of this complex disease is depicted in Figure 1. The initiation of atherosclerosis is the damage to the endothelium of an artery. Principal risk factors include elevated levels of LDL-C, hypertension, diabetes, and smoking (Betteridge, 1987; Leaf & Weber, 1988). With an endothelial injury, platelets subjected to the underlying collagen are activated, resulting in the synthesis of thromboxane and platelet aggregation. Platelet-derived and other growth factors are released which cause smooth muscle cells in the arterial intima to migrate to the site of the endothelial injury and proliferate (Dodson & Horton, 1987; Leaf & Weber, 1988).

Due to the hydrophobicity of lipid, it was postulated that it entered the arterial wall as lipoproteins. Both LDL and apoprotein B (apo B) of LDL have been found in atherosclerotic lesions in direct proportion to plasma levels (Dodson & Horton, 1987). The LDL in the lesions may be altered by free radicals of oxygen and phospholipase A_2. The smooth muscle cells and macrophages then ingest the circulating lipids by means of the non-LDL receptor scavenger pathway to become foam cells. The accumulation of cholesterol is also promoted by platelets. The lesions, adherent thrombi, collagen and necrosis with calcium deposits enlarge over a

Figure 1. Pathogenesis of Atherosclerosis and Thrombosis in the Artery. Adapted from Leaf and Weber, 1988.



period of time and eventually constrict the arterial lumen. The overlying endothelium is susceptible to further injury, creating a vicious cycle. A thrombus eventually obstructs the artery, causing myocardial infarction, which may result in fatal arrhythmias (Betteridge, 1987; Dodson & Horton, 1987; Leaf & Weber, 1988; Ross, 1986).

1.3 Dietary Fatty Acids and Atherosclerosis

1.3.1 LDL-C and Risk of CHD

Hypercholesterolemia or elevated levels of LDL-C and its association with the risk of CHD have been reported in numerous epidemiological (Castelli et al., 1986; Goldbourt & Yaari, 1990; Kannell et al., 1984; Keys, 1970), clinical (Grundy, 1986; Lipid Research Clinics Program, 1984) and animal studies (Ross, 1986 ; Weiner et al., 1986). On the other hand, HDL-C level is inversely related to the rates of CHD (Castelli et al., 1986; Goldbourt & Yaari, 1990).

A major epidemiological study that examined the associations among diet, plasma cholesterol, other risk factors and CHD rates in different populations was the Seven Countries Study. The dietary factor most closely correlated with higher levels of plasma cholesterol and CHD rates was the level of SFA intake. The population which consumed olive oil (rich in OA) as a major fat source had very low CHD rates (Keys, 1970). This leads to the question as to how dietary fatty acids affect cholesterol metabolism which in turn

affects the rates of CHD.

1.3.2 Regulation of LDL Metabolism and Hypercholesterolemia

The discovery of the cell-surface LDL receptors was fundamental to the understanding of the regulation of LDL metabolism (Brown et al., 1981; Brown & Goldstein, 1984; Grundy, 1987; Grundy & Vega, 1990). The basic steps in the metabolism of LDL are outlined in Figure 2. The LDL are the primary cholesterol-bearing lipoproteins of plasma. They are rich in cholesteryl esters (CE) and have very little triglyceride (TG).

The main mechanism for clearance of LDL is the LDL receptor situated on the surface of hepatocytes (Brown & Goldstein, 1984; Grundy & Vega, 1990). LDL receptors bind circulating LDL and the receptor-LDL complex is transferred into lysosomes of cells where the degradation of LDL occurs. Approximately 70% of circulating LDL is cleared via the receptor pathway while the rest is removed via a non-specific non-receptor pathway. Thus, LDL receptor activity is the key factor in the determination of plasma LDL levels (Brown et al., 1981; Brown & Goldstein, 1984; Grundy, 1987; Grundy & Vega, 1990).

Usually an increase in plasma cholesterol is due to an elevated level of LDL. As the LDL level increases, so does the risk for CHD. The primary cause of a high level of LDL is a reduction in the activity of LDL receptors (Figure 3). When

Figure 2. Regulation of LDL Metabolism. Adapted from Grundy, 1987; Grundy & Vega, 1990.

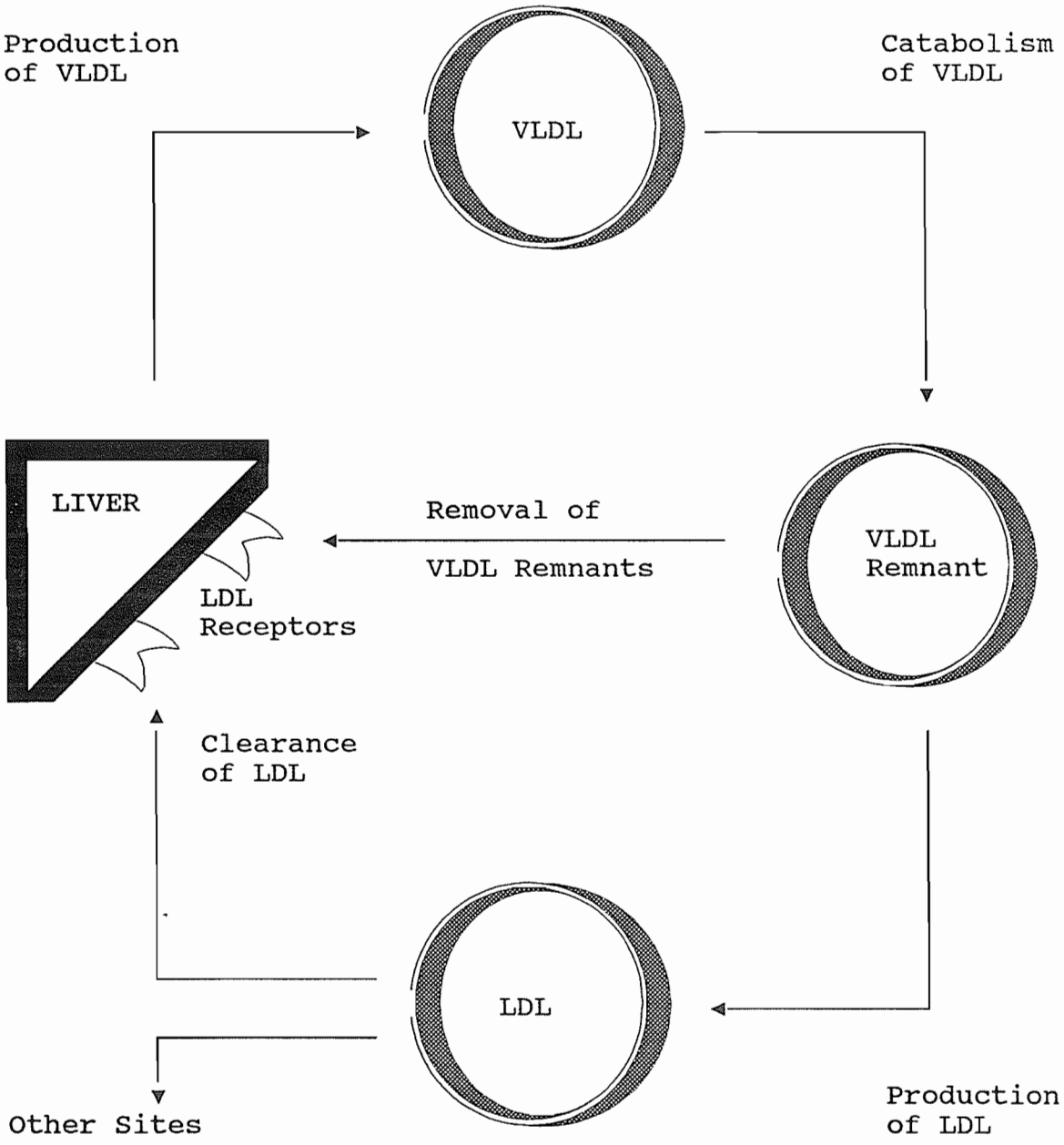
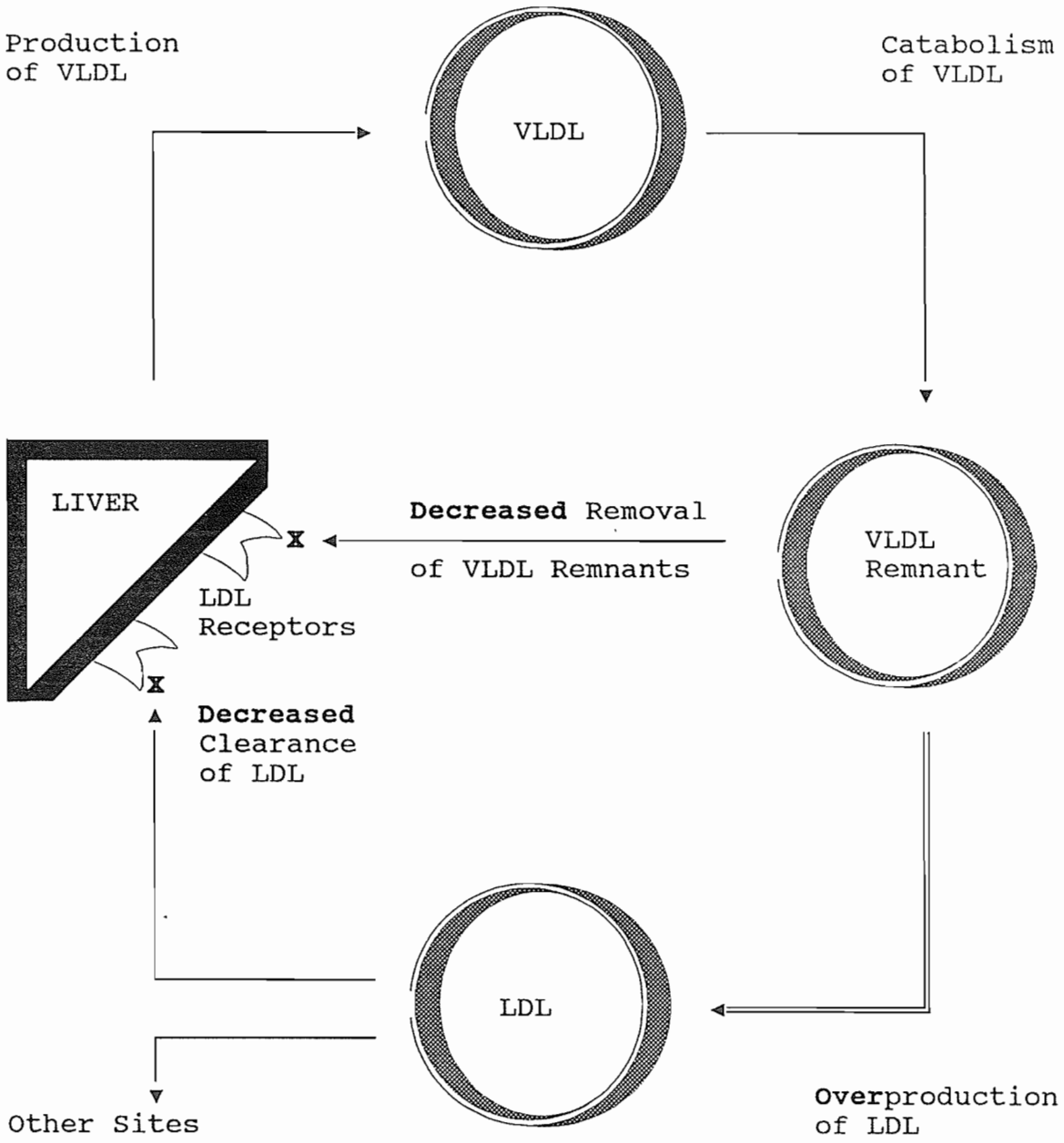


Figure 3. Metabolic Consequences of a Decrease in the Activity of LDL Receptors. Adapted from Grundy, 1987; Grundy & Vega, 1990.



the number of receptors is decreased, the removal of LDL is decreased and because VLDL remnants also are cleared via LDL receptors, more VLDL remnants are converted to LDL. One in 500 people has an abnormal gene for LDL receptors and thus the number of functioning LDL receptors is only half normal. This condition is known as heterozygous familial hypercholesterolemia (FH) (Grundy, 1987; Grundy & Vega, 1990; Motulsky, 1989). Individuals with FH are prone to develop CHD. However, not all individuals with cholesterol levels greater than 6.2 mmol/L have FH, indicating that both genetic and dietary factors are probably involved. Most hypercholesterolemic individuals have a reduced activity of LDL receptors, although they do not have a primary defect in the structures of the genes encoding for LDL receptors (Grundy, 1987; Grundy & Vega, 1990, 1985).

The synthesis of LDL receptors is controlled by a feedback mechanism (Brown et al., 1981). For instance, when levels of cholesterol in hepatocytes increase, the synthesis of LDL receptors is suppressed and plasma levels of LDL increase. Thus, any factors that will increase the content of cholesterol in the hepatocytes should suppress the LDL receptor activity. Excess hepatic cholesterol may be due to an inherited hyperabsorption of cholesterol or a defect in the conversion of cholesterol into bile acids in the hepatocytes. Another factor that appears to modulate the LDL receptor activity is diet. Two dietary components that suppress LDL

receptor activity are SFA and cholesterol. Dietary cholesterol suppresses LDL receptor activity by increasing the hepatic content of cholesterol, and SFA appear to act via a similar mechanism, i.e. inhibit receptor-mediated uptake of LDL (Applebaum-Bowden et al., 1984; Connor & Connor, 1989; Grundy, 1987; Grundy & Vega, 1990).

1.3.3 Effects of Dietary Fatty Acids on Plasma Lipids and Lipoproteins

As mentioned earlier in section 1.3.1, the dietary factor most closely correlated with higher levels of plasma cholesterol and CHD rates was the level of SFA consumption, and the population which consumed high levels of OA had very low CHD rates (Keys, 1970). According to the equations of Keys et al. (1957) and Hegsted et al. (1965), OA had no effect on the plasma cholesterol level and SFA raised the TC about twice as much as LA lowered it. However, studies done more recently showed that OA did have an effect on cholesterol metabolism. For instance, Grundy (1986) found that as compared to SFA, OA lowered plasma TC and LDL-C. Similarly, Mensink and Katan (1987) found that an olive oil-rich diet caused a significant fall in TC and LDL-C levels while HDL-C level remained unchanged.

Becker et al. (1983), studying the effects of dietary fat source on plasma cholesterol and lipoproteins, found that LA lowered TC and LDL-C to a greater extent than OA. By

contrast, Mattson and Grundy (1985), comparing the effects of OA and LA, found both fatty acids to be as effective in lowering TC and LDL-C. However, some investigators reported that LA also lowered HDL-C, especially when it was consumed in large quantities (Mattson & Grundy, 1985; Shepherd et al., 1980; Vega et al., 1982). The reduction in HDL-C levels by LA has been of concern since HDL-C levels are inversely related to the risk of CHD. Hence, more research has been carried out to study the effects of dietary fatty acids on plasma lipids and lipoproteins.

Baggio et al. (1988), studying the effect of olive oil on lipoproteins with a group of mildly hypercholesterolemic subjects, found that olive oil, which is rich in OA, was effective in lowering TC and LDL-C while HDL-C level remained constant. A number of recent studies indicated that OA and LA were equally effective in lowering TC and LDL-C without affecting HDL-C (Chan, 1990; Masana et al., 1991; McDonald et al., 1989; Mensink & Katan, 1989; Wardlaw & Snook, 1990). These studies, however, used only normolipidemic subjects. The effects of these fatty acids on the cholesterol metabolism of hypercholesterolemic subjects are still unclear. This is because of the fact that there is relatively little information available on the lipoprotein responses to these fatty acids in individuals with or without genetic conditions of hypercholesterolemia.

Sirtori et al. (1986), in examining the effects of olive

oil and corn oil on plasma lipids of patients with clinical hyperlipidemia, found that plasma TC was reduced with corn oil, but HDL-C levels were lower with corn oil and unchanged or raised by olive oil. Since corn oil is high in LA and olive oil is high in OA, the results showed that OA and LA were not equally effective in influencing cholesterol metabolism in the hyperlipidemic patients. A recently published study by Friday et al. (1991) showed that FH and normal individuals responded similarly to diets low in SFA and rich in n-6 and n-3 PUFA with decreased LDL-C and apo B levels. Also, plasma TG and HDL-C dropped significantly with n-3 diets in normal and FH individuals. The n-3 PUFA in this case was salmon oil. The effect of n-3 PUFA of plant origin on cholesterol metabolism is of current interest because of its potential effect on thrombosis.

The effect of LNA on cholesterol metabolism has not been studied as extensively as those of SFA, OA, and LA. Inconsistent changes in plasma cholesterol levels were reported by Sanders and Roshanai (1983) and Mest et al. (1983), where subjects were given linseed oil supplements, which were high in LNA, although the results may have been compromised by a failure to control dietary fat content in these studies. Chan (1990) in our laboratory found that OA, LA and LNA were equally hypocholesterolemic, without affecting HDL-C levels in a group of normolipidemic individuals. It would be interesting to study the effects of these fatty acids

on cholesterol metabolism in a group of hyperlipidemic subjects.

Although the hypocholesterolemic effect of OA and LA is quite clear with respect to normolipidemic subjects, the mechanisms for this hypocholesterolemic effect have not been clearly demonstrated. Spady and Dietschy (1982) suggested that SFA suppress receptor-mediated clearance of LDL, and this seems to explain their action in elevating plasma LDL levels. Thus, when OA and LA replace SFA, the decrease in plasma LDL levels may merely reflect the removal of receptor-suppressing SFA. Mattson and Grundy (1985) proposed the following explanations: the major effect of OA and LA when they replace SFA is to decrease the level of LDL. The possible mechanisms are (i) by reducing the production of VLDL, the precursor of LDL, (ii) by decreasing the cholesterol content of LDL fractions, and (iii) by enhancing the activity of LDL receptors. However, the most convincing mechanism is the enhancement of LDL receptor activity. The hypocholesterolemic effect of LNA, although this needs to be confirmed, may or may not be acting via similar mechanisms as described above.

1.4 Dietary Fatty Acids and Thrombosis

1.4.1 The Eskimo Experience

The effect of fish oil on thrombosis has been given lots of attention in previous research. One of the well-known studies was on Greenland Eskimos. It was found that the

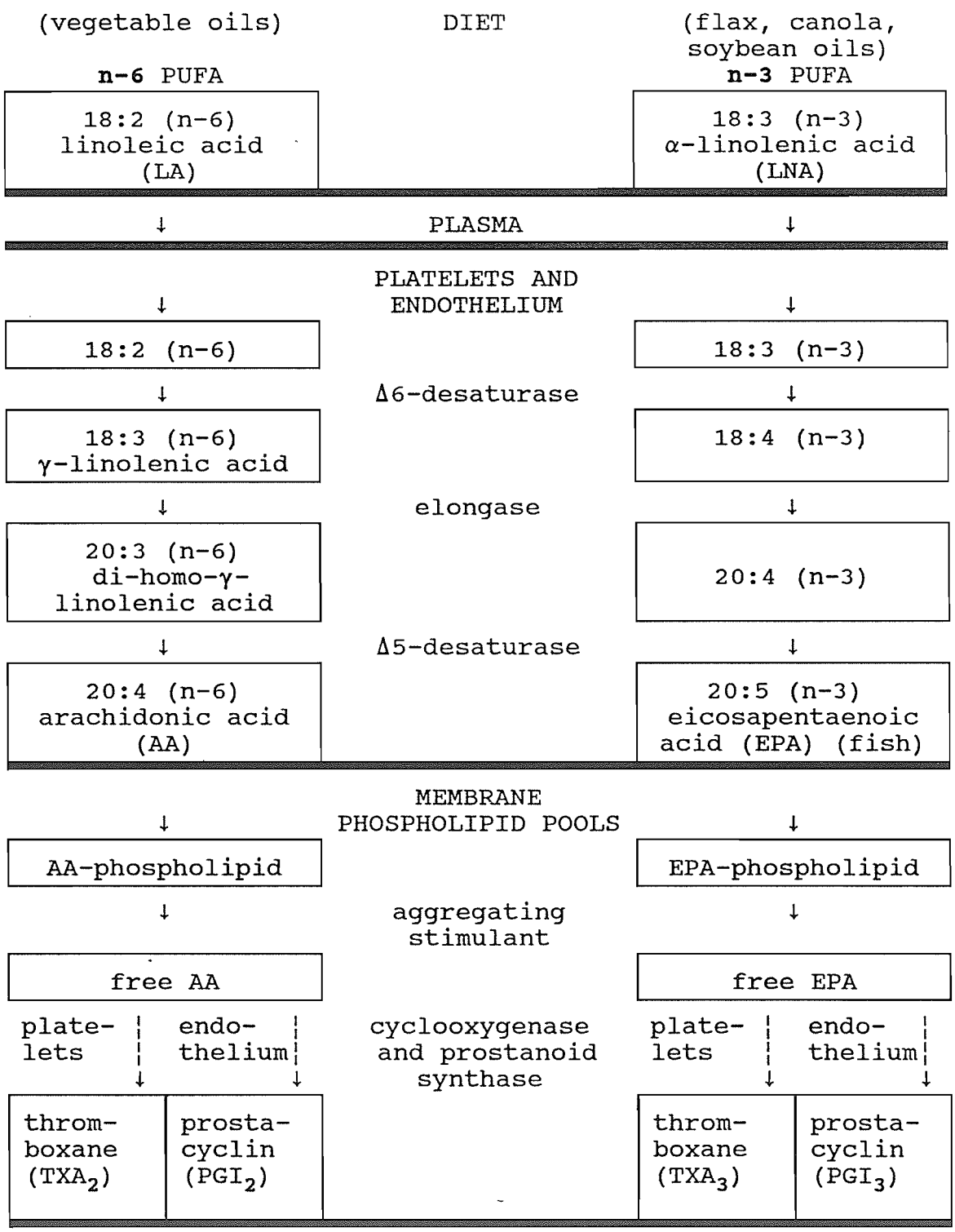
Eskimos experienced a lower incidence of CHD than the Danes. The total amount of fat consumed by Eskimos was similar to that consumed by Danes. However, Eskimos consumed a higher amount of EPA. It was also observed that the Eskimo platelet lipids were enriched with EPA and this was associated with prolonged bleeding time and reduced platelet aggregation by collagen and adenosine diphosphate (ADP) compared with those of Danes and Eskimos living in Denmark (Dyerberg, 1986; Dyerberg & Bang, 1979; Nordoy & Goodnight, 1990).

Fish oil is rich in EPA and docosahexaenoic acid (DHA). EPA is proposed to be the precursor of antithrombogenic prostanoid, which will be discussed in the next section. The function of DHA is not clearly known and it has been reported to be present in high amounts in the brain and retina (Cook, 1985; Dupont, 1990; Leaf & Weber, 1988; Sanders & Rana, 1987). Also, DHA may act as a potential storage form of EPA (Leaf & Weber, 1988).

1.4.2 Proposed Mechanisms of Prostanoid Synthesis

The proposed mechanisms of prostanoid synthesis are depicted in Figure 4. Briefly, LA and LNA share the same enzyme systems in the desaturation and chain elongation pathways. Since LNA is the preferred substrate over LA, which in turn is the preferred substrate over OA by the rate-limiting Δ6-desaturase (Cook, 1985; Dupont, 1990, 1987; Kinsella, 1988; Mead et al., 1986; Sanders & Rana, 1987), the

Figure 4. Proposed Pathways of Prostanoid Synthesis. Adapted from Cook, 1985; Dupont, 1990; Kinsella, 1988; Kinsella et al., 1990; Leaf and Weber, 1988.



pathway of OA will not be discussed.

LA is eventually converted to arachidonic acid (AA) which is the precursor of TXA_2 and PGI_2, while LNA gives rise to EPA which is the precursor of TXA_3 and PGI_3. Both PGI_2 and PGI_3 are vasodilating and antiaggregatory, whereas TXA_2 is vasoconstricting and proaggregatory and TXA_3 is a very weak platelet aggregator (Dupont, 1990, 1987; Holub et al., 1987; Kinsella, 1988; Leaf & Weber, 1988; Mead et al., 1986). Therefore, if LNA competes favourably for the rate-limiting Δ6-desaturase and gives rise to more EPA in the body, then the shift of the prostanoid homeostasis will be towards a less thrombogenic state.

AA can be further elongated to docosatetraenoic acid (DTA) while EPA can be further converted to docosapentaenoic acid (DPA) and eventually to DHA. The importance of these further elongated and desaturated PUFA is in the proper functioning of the brain and retina (Cook, 1985; Dupont, 1990; Leaf & Weber, 1988; Sanders & Rana, 1987).

1.4.3 Beneficial and Adverse Effects of EPA

As mentioned previously, one of the most significant studies was that concerning the Greenland Eskimos carried out by Dyerberg and Bang in the 1970s. The high-EPA diet of the Eskimos was associated with prolonged bleeding time and reduced platelet aggregation when compared with those of Danes and Eskimos living in Denmark. According to Dyerberg (1986),

although the prostaglandin hypothesis, based on the formation of TXA_3 and PGI_3, and the inhibition of TXA_2 synthesis was tempting, it did not explain all their findings.

Weiner et al. (1986), studying the effect of cod liver oil on the progression of CHD in a hyperlipidemic swine model, found that platelet AA was markedly reduced, platelet EPA was increased, and plasma TXA_2 was decreased in the oil-fed group as compared with the control group. They concluded that the dietary EPA retarded the progression of CHD possibly through changes in prostaglandin metabolism.

A number of studies on the effect of abundant fish oil consumption were reported to cause significant increases in the levels of EPA and DHA in plasma and platelet lipids. Plasma and platelet levels of LA and AA either decreased or remained unchanged. Reductions in ADP- and collagen-induced platelet aggregation and prolonged bleeding times have been frequently observed. Other observations included the concomitant reduction and formation of TXB_2 and TXB_3, respectively. Also, the human smooth muscle cells readily converted EPA to PGI_3 while PGI_2 remained unchanged. The results indicated that high fish oil consumption has an effect on prostanoid metabolism, causing a decrease in thrombosis (Herold & Kinsella, 1986). Meanwhile, some authors (Dyerberg, 1986; Kinsella, 1988; McNamara, 1990) raised concerns regarding possible adverse effects of high EPA consumption.

Dyerberg (1986) and Kinsella (1988) expressed concern

with the ad libitum intake of cod liver oil because of the potential toxicity of vitamins A and D, increased lipid peroxidation or carcinogenesis, and decreased inflammatory and immune responses. As a supplement to a low-SFA, low-cholesterol diet, fish oil capsules are not recommended for hypercholesterolemic (Dart et al., 1989; McNamara, 1990) or hypertriglyceridemic individuals (Inagaki & Harris, 1990; Radack et al., 1990). Although these concerns are legitimate, the definitions of optimum and excess amounts of EPA have not been clearly defined in the literature.

1.4.4 LNA Metabolism and Thrombosis

Some vegetable oils are rich in LNA, such as flax, canola and soybean oils, and these are wholesome and accessible alternatives to fish oil. However, unlike EPA, LNA which is the parent compound of the n-3 PUFA has not been studied extensively. LNA deserves our attention because, as discussed earlier, it competes favourably against LA such that the conversion of LA to AA is inhibited. This, in turn, decreases the synthesis of prothrombogenic TXA_2. Furthermore, LNA is the precursor of EPA, thereby producing antithrombogenic prostanoids. There has been some evidence from animal and human studies indicating effects of dietary LNA on prostanoid metabolism as well as on platelet function.

1.4.4.1 Studies in Animals

A recent study with rats fed diets high in linseed, sunflower or fish oils for three weeks found that the content of AA in liver phospholipids (PL) increased with diets rich in LA, and was decreased for the diet rich in EPA and DHA. On the other hand, the amount of EPA in PL was as high with the linseed oil diet as with the fish oil diet, while DHA content was only increased with the fish oil diet (Christiansen et al., 1991). An earlier study by Ishinaga et al. (1983) with rats fed diets containing linseed oil, safflower oil or cocoa butter for 27 weeks showed that comparing with the other diets, the linseed oil diet markedly decreased the AA level in platelet phosphatidylcholine (PC) and accumulated the EPA in all PL. The collagen-induced aggregation of washed platelets decreased only in rats fed linseed oil. These findings suggested that the feeding of linseed oil, which has a high LNA content, alters the fatty acid composition of platelet PL similarly to the feeding of fish oils, and thereby reduces the aggregation tendency of platelets.

Similar results were obtained by Marshall and Johnston (1982) with rats fed diets containing ratios of LA/LNA of 32/1, 7/1, 1/1 and 0.3/1 in the form of corn oil, soybean oil, soybean/linseed oil mix and linseed oil for two months. As the level of LNA in the diet increased, AA content of liver and spleen phosphatidylethanolamine (PE) decreased while EPA in the same fractions increased. The replacement of the n-6

PUFA by the n-3 PUFA is explained by the effective competition of LNA over LA for the Δ6-desaturase.

1.4.4.2 Studies in Humans

Again, most of the studies on LNA metabolism and thrombosis were conducted using healthy subjects. Most studies were short-term (Adam et al., 1986; Budowski et al., 1984; Mest et al., 1983; Sanders & Roshanai, 1983) while a study by Renaud et al. (1986) is a good example of a long-term study.

Adam et al. (1986) investigated the effect of dietary LNA intake on LA metabolism in two groups of six healthy women. The diets containing a constant amount of LA and varying amounts of LNA from 0% to 16% of calories were given for two weeks each. It was found that the levels of EPA increased in all plasma and platelet lipids with augmented LNA intake, suggesting a chain elongation and desaturation of LNA to EPA. On the other hand, AA levels in all plasma and platelet lipids remained constant with increased LNA intake. Similar findings were observed by Sanders and Roshanai (1983) when five healthy individuals were supplied with 20 ml/day linseed oil for two weeks. After a six-week break, the same subjects consumed a similar amount of MaxEPA (fish oil concentrate) for two weeks. The EPA content in platelet lipids was increased by both supplements although the increase brought about by the linseed oil was small. The platelet AA content was decreased by

MaxEPA but not by the linseed oil supplement.

By contrast, studies by Mest et al. (1983) and Budowski et al. (1984) indicated that LNA did have an effect on LA metabolism in humans. Mest et al. (1983) investigated the effect of 30 ml/day linseed oil supplement in ten healthy subjects over a four-week period. The levels of LNA, EPA and DHA increased while those of LA and AA decreased in plasma PL after supplementation with linseed oil. However, thromboxane synthesis in platelets was unchanged. In the study by Budowski et al. (1984), the eight normolipidemic volunteers had a 60 ml/day linseed oil supplement over a period of six weeks. Plasma LNA and EPA increased, whereas LA and AA decreased. A striking decrease in platelet sensitivity to collagen was observed after supplementation with linseed oil. The findings show that linseed oil, which is rich in LNA, affects plasma PUFA metabolism and platelet aggregability. The results coincide with the findings of Renaud et al. (1986) from a long-term study of French farmers, in which a three-fold increase in the consumption of LNA caused a considerable alteration in platelet function, in spite of only minor changes in plasma and platelet lipids.

Briefly, Renaud et al. (1986) investigated the effect of a long-term diet modification on platelet function in a group of 98 French farmers. The diet of one group of farmers was modified by increasing the LNA consumption from 0.37% of calories to 1.00% of calories for one year. This resulted in

significantly higher levels of EPA in plasma and platelet lipids which, in turn, improved the clotting activity of platelets and aggregation to thrombin and collagen but not to ADP. These studies used different amounts of LNA in the experimental diets and this arouses the interest in the importance of dietary LA/LNA ratio in thrombosis.

1.4.5 Importance of Dietary LA/LNA Ratio in Thrombosis

As discussed in section 1.4.2, there is a considerable competition between LA and LNA for the same enzyme systems in the fatty acid desaturation and chain elongation process. Cook (1985) indicated that LNA is a preferred substrate to LA for the Δ6-desaturase enzyme. However, in the study by Adam et al. (1986), both plasma and platelet PL AA levels remained constant when the LA/LNA ratio of the diet was decreased to 0.25/1. In other words, the conversion of LA to AA was not suppressed by the ingestion of LNA. This raises the issue as to whether LA/LNA ratio does have an effect on n-6 and/or n-3 PUFA metabolism.

Since Western conventional diets have a higher level of LA than LNA, LA may compete favourably for the Δ6-desaturase enzyme and hence, decrease the formation of EPA, the precursor of antithrombogenic prostanoids. Thus, by decreasing the intakes of LA or by lowering the LA/LNA ratio, prostanoid homeostasis may be shifted towards a less thrombogenic state.

A study on vegetarians and omnivores by Sanders and

Younger (1981) demonstrated the importance of a low LA/LNA ratio. They administered linseed oil supplements such that the supplement reduced the LA/LNA ratio from 6/1 to 1/1 in the omnivores, which resulted in a two-fold increase in the level of EPA in plasma PC (1.3% to 2.7%) while the same supplement, which reduced the LA/LNA ratio from 16/1 to 3/1 in the vegetarians, resulted in a three-fold increase in the level of EPA in plasma PC (0.3% to 1.0%). Similar results were obtained by Corner et al. (1990) and Weaver et al. (1990) when investigating the effect of dietary LA/LNA ratios of 2.6/1 and 73.9/1 on PUFA metabolism in normolipidemic subjects. EPA levels were significantly higher in plasma (Corner et al., 1990) and platelet PC and alkenylacyl ethanolamine phosphoglyceride (PPE) fractions (Weaver et al., 1990) after a diet of low LA/LNA ratio. By contrast, a low dietary ratio caused a lowering of AA levels in plasma PE and PPE (Corner et al., 1990) and platelet PC fractions (Weaver et al., 1990).

Similar results were observed by Chan (1990) with a group of normolipidemic subjects. It was observed that diets with LA/LNA ratios of 3/1 and 2.7/1 produced similar increases in plasma EPA levels. By contrast, diets high in LA and LNA were usually associated with significantly lower levels of eicosatrienoic acid (ETA) and total n-6 PUFA in the plasma. The lower plasma ETA and total n-6 PUFA levels found following the diets high in LA and LNA could be due to the inhibitory effect of LNA on the n-6 PUFA metabolism (Ziboh &

Chapkin, 1988). It was deduced that the absolute amount of LNA may have an important influence on the n-6 PUFA metabolism while the LA/LNA ratio may be more important in influencing the n-3 PUFA metabolism. Since the study conducted last year in our laboratory (Chan, 1990) was with a group of normolipidemic subjects, it would be interesting to see if the absolute and relative amounts of dietary LA and LNA would have the same impact on the n-6 and n-3 PUFA metabolism in a group of hyperlipidemic individuals.

1.5 Background Rationale

CHD is the leading cause of death in western industrialized nations such as Canada. It is caused by two major events, namely, atherosclerosis and thrombosis. The hypercholesterolemic effect of SFA has been very well established. The hypocholesterolemic effect of OA and LA also has been clearly demonstrated. However, the cholesterol-lowering effect of LNA needs further clarification.

Furthermore, LA and LNA can be desaturated and elongated to AA and EPA, respectively, by the same enzyme systems. AA is the precursor of the vasoconstricting and proaggregatory TXA_2 and antiaggregatory and vasodilating PGI_2, while EPA is the precursor of a very weak platelet aggregator, TXA_3 and antiaggregatory and vasodilating PGI_3. Therefore, if LNA competes favourably for the rate-limiting Δ6-desaturase and gives rise to more EPA in the body, then the shift of the

prostanoid homeostasis will be towards a less thrombogenic state. This optimism has led many researchers to look into the importance of dietary LA/LNA ratio in thrombosis (Chan, 1990; Corner et al., 1990; Sanders & Younger, 1981; Weaver et al., 1990). It is possible that the levels of LA and LNA are of significance as well in prostanoid homeostasis.

Holub et al. (1988) found that the major reservoirs of the long chain PUFA, i.e. AA, EPA, DPA and DHA in platelet PL are in the PC, PE and PPE fractions. Also, Laustiola and Salo (1986) and Joist et al. (1976) reported that although there is no direct evidence to show that prostanoid precursor fatty acids found in plasma are used as substrates for prostanoid synthesis, it has been shown in vitro that plasma and platelets may exchange PL. Thus, assuming a similar distribution of fatty acids occurs in the plasma PL, the present study focused on the fatty acid patterns in the respective plasma PL fractions. Furthermore, a direct relationship exists between the fatty acid patterns of PC and CE, since it has been demonstrated that lecithin : cholesterol acyltransferase catalyses the transfer of PUFA from the 2-acyl position of PC to free cholesterol to give rise to CE in vivo (Holub et al., 1987; Mead et al., 1986). Hence, the fatty acid patterns of CE also were examined in the present study.

The study by Chan (1990) suggested that the absolute amount of dietary LNA may be more important in influencing n-6 PUFA metabolism while the LA/LNA ratio may be more important

in influencing n-3 PUFA metabolism in a group of normolipidemic subjects. Thus, it would be interesting to see if the absolute and relative amounts of dietary LA and LNA would have the same impact on the n-6 and n-3 PUFA metabolism in a group of hyperlipidemic individuals.

2. OBJECTIVES

The primary objectives of the current study were to investigate the effect of diets with varying amounts of SFA, OA, LA and LNA on plasma lipid and lipoprotein metabolism and on the fatty acid patterns of plasma PL, viz., PC, PE and PPE, and CE in a group of individuals with elevated cholesterol levels (≥5.8 mmol/L). From these observations, it should be possible to deduce some implications with respect to: (i) the effect of dietary SFA, OA, LA and LNA on plasma lipid and lipoprotein metabolism; and (ii) the effect of absolute and relative amounts of dietary LA and LNA on the fatty acid patterns of plasma PL and CE in this group of subjects.

3. MATERIALS AND METHODS

3.1 Experimental Design

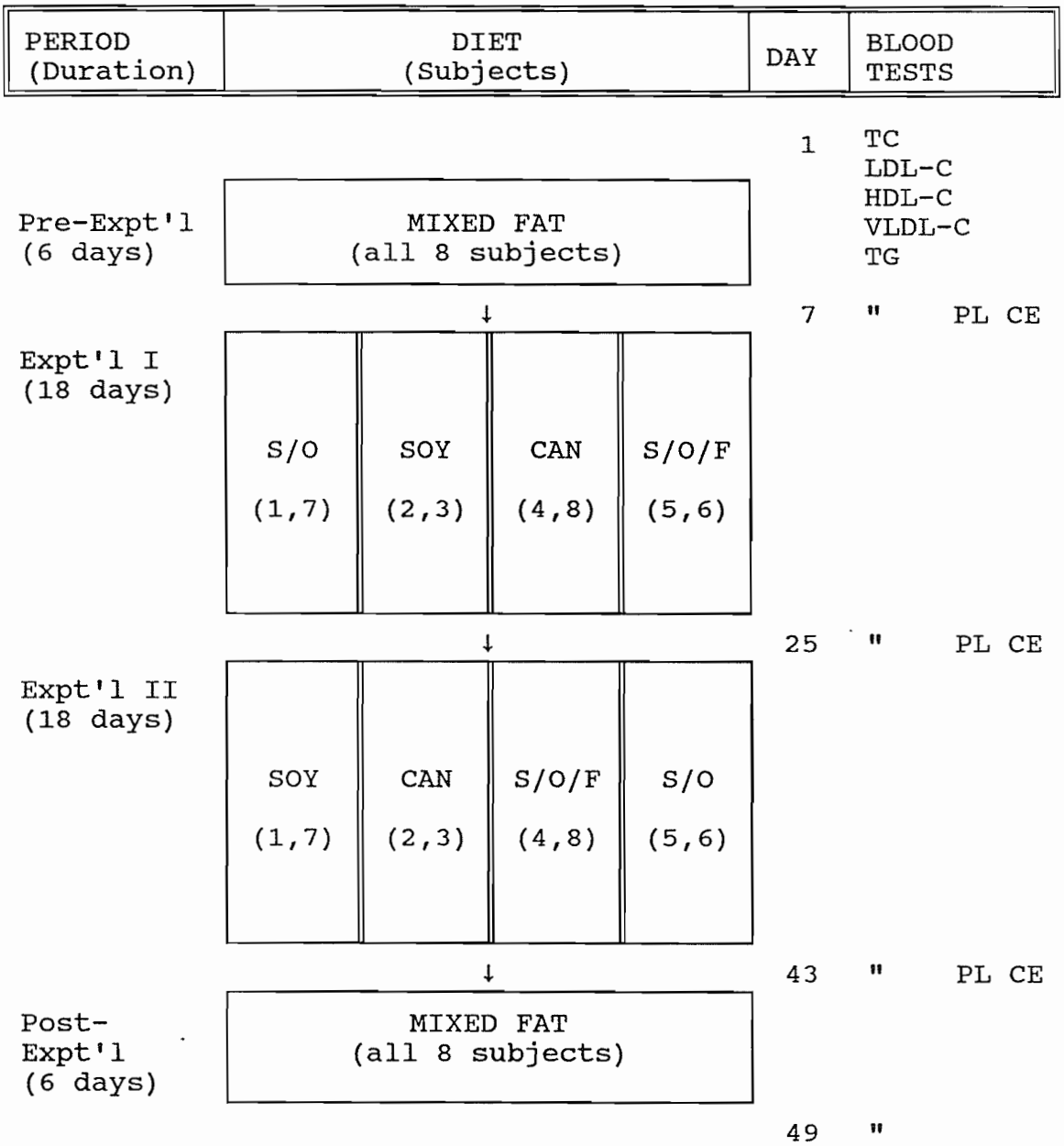
The study was comprised of two 48-day replicates. Each replicate was divided into four periods: a 6-day pre-experimental period, two 18-day experimental periods and a 6-day post-experimental period (Figure 5). There was about a two-month gap between replicates. The eight subjects were initially divided into two groups on the basis of their TC levels on Day 1. They received the mixed fat diet during the pre- and post-experimental periods. In the first experimental period, two subjects, one from each group, were randomly assigned to one of the experimental diets: sunflower and olive diet (S/O), canola diet (CAN), soybean diet (SOY), sunflower, olive and flax diet (S/O/F). Consequently, each group had a subject with a higher TC level and a subject with a lower TC level. In the second experimental period, each pair of subjects was switched to a different diet according to the following order: S/O to SOY, SOY to CAN, CAN to S/O/F, S/O/F to S/O.

Venous blood samples were drawn from each subject after a 12-hour overnight fast on Days 1, 7, 25, 43 and 49 of each replicate.

3.2 Subjects

A rapid screening for TC was set up to pre-select individuals with elevated plasma cholesterol levels using the

Figure 5. Experimental Design (REPLICATE I)[1, 2]



[1] The gap between replicates was 65 days.
[2] For REPLICATE II, the subjects in S/O, SOY, CAN & S/O/F diet sequences were subjects 12, 11 & 15, 10 & 14, and 9 & 13, respectively.

Vision System Analyzer (Courtesy of Mr. Rick Barker, Abbott Laboratories). Those with TC ≥ 6.0 mmol/L were invited to have their TC checked again, following a 12-hour fast,before being invited to participate in the study. The purpose, nature and restrictions associated with the study were explained to the potential subjects. Those willing to comply with all the specifications associated with the study were invited to become subjects. The subjects were also required to undergo a medical examination prior to inclusion in the study.

Thirteen male volunteers were recruited for the study. There were eight subjects in the first replicate and seven subjects in the second replicate. Two subjects participated in both replicates. All subjects were students of the University of Manitoba. Only two of the subjects had a family history of CHD. None of the subjects were taking any medications. The physical data for the subjects, who ranged from 18 - 38 years in age,is shown in Table 1.

The protocol of the study was approved by the Faculty of Human Ecology Ethics Committee and written consent was obtained from each subject. A copy of the consent form used in the study is shown in Appendix 1.

Consumption of alcohol was prohibited. Subjects were instructed against the use of aspirin and were informed to consult with the project directors before taking any medications. The subjects maintained their usual activity

Table 1. Physical Data for the Subjects.

Subject[1]	Height	Initial Weight	BMI[2]	Plasma Cholesterol	
				Screening[3]	Fasting[4]
	(cm)	(kg)	(kg/m²)	(mmol/L)	(mmol/L)
1	183.0	83.5	24.9	5.9	5.7
2	180.0	72.2	22.3	8.0	7.1
3[5]	160.0	60.4	23.6	6.4	6.3
4	185.0	83.0	24.3	5.9	5.6
5[5]	175.0	76.0	24.5	6.4	6.3
6	176.0	61.0	19.7	6.3	5.8
7	175.0	84.1	27.5	7.3	6.7
8	162.0	73.5	28.0	7.2	6.7
9	167.5	92.4	32.9	6.7	6.7
10	191.3	105.1	28.7	5.9	6.0
11	172.5	73.1	24.6	6.5	6.2
12	170.0	69.0	23.9	6.2	5.9
13	175.0	77.0	25.1	6.2	6.5
14[5]	160.0	60.4	23.6	6.3	5.6
15[5]	175.0	76.0	24.5	5.9	6.0

[1] The first 8 subjects participated in Replicate I of the study.
[2] Body Mass Index.
[3] Measured about 3 weeks prior to the start of the study.
[4] Measured about 2 weeks prior to the start of the study.
[5] The two subjects who participated in both Replicates I & II.

patterns and resided in their own places of residence throughout the study.

3.3 Diets

The five diets, which differed only in sources of added fat, were designed to be nutritionally adequate. A two-day cyclic menu of conventional foods is shown in Table 2. The diets were designed to provide approximately 3200 kcal/day, of which 51% of energy was contributed by CHO, 13% by protein and 36% by fat (Table 3).

The added dietary fat accounted for approximately 81% of total dietary fat (29% of total energy). During the pre- and post-experimental periods, the added fat was made up of a mixture of fats similar to the average consumption in Canada (mixed fat diet). During the experimental periods, the added fat sources were: (i) a mixture of sunflower and olive oils (S/O diet), (ii) canola oil (CAN diet), (iii) soybean oil (SOY diet), and (iv) a mixture of sunflower, olive and flax oils (S/O/F diet). The composition of the added fat in each diet is outlined in Table 4. The distribution of the added fat among the various foods in the diets is shown in Table 5.

The saturated, oleic, linoleic and linolenic acids composition of the diets is shown in Table 6. The fatty acid composition of the oils, diets and the contribution of SFA, OA, LA and LNA in the different diets to total energy intake are presented in Appendices 2, 3 and 4, respectively. The

Table 2. Two-Day Cyclic Menu[1]

	Food Item (Amount)	
	Day I	Day II
Breakfast	Orange juice (125 ml) Granola[2, 3] Skim milk (125 ml)	Orange juice (125 ml) Granola[2, 3] Skim milk (125 ml)
Lunch	Cold chicken (60 g)[4] Tomatoes (100 g) Lettuce (20 g) Oil[2] & vinegar Rice[2, 3] Jellied fruit[3] Skim milk (250 ml)	Chili[2, 3, 5] Lettuce (20 g) Oil[2] & vinegar Jellied fruit[3] Skim milk (250 ml)
Dinner	Hamburger patty[5] (60 g cooked) Mashed potatoes[2, 3] Carrots[2, 3] Canned pears (120 g) Skim milk (250 ml)	Chicken casserole[2,3,4] Noodles[2, 3] Canned peaches (120g) Skim milk (250 ml)
Snacks	Raw apple (1 medium) Cookies[2, 3] (4) Skim milk (125 ml)	Raw apple (1 medium) Cookies[2, 3] (4) Skim milk (125 ml)
Bread	8 slices	7 slices
Jam	2 packages (28.4 g)	2 packages (28.4 g)
Diet jam	2 packages (28.4 g)	2 packages (28.4 g)
Spread[2]	1 package	1 package

[1] Coffee, tea, diet soft drinks and artificial sweetener were allowed ad libitum.
[2] See Table 5 for the amounts and types of oil or fat added to the items in different diets.
[3] See Appendix 5 for recipes.
[4] Skinless chicken breast.
[5] Top round, ground beef.

Table 3. Macronutrient Content of the Diets[1]

Nutrient	Weight (g)	% of Total Energy[2]
Carbohydrate	418	51
Protein	107	13
Fat	129	36

[1] Means of 20 1-day duplicate diet samples.
[2] Total energy intake was 3260 kcal.

Table 4. Sources of Added Dietary Fat[1]

Diet	Fat Source
Mixed Fat[2]	11% Corn oil 22% Lard 22% Tallow 22% Shortening 23% Butter
S/O[3]	20% Sunflower oil 80% Olive oil
CAN[4]	100% Canola oil
SOY[5]	100% Soybean oil
S/O/F[6]	47% Sunflower oil 20% Olive oil 33% Flax oil

[1] Accounted for approximately 81% of total dietary fat. The remaining 19% was contributed by invisible fat present in foods used in the diet and by approximately 11 g of butter fat present in each diet.

[2] Mazola Corn Oil, Best Foods Division, Canada Starch Co. Ltd., Montreal, PQ. Tenderflake Lard, Canada Packers Ltd., Toronto, Ont. Tallow, Canada Packers Ltd., Winnipeg, Man. Crisco Shortening, Procter and Gamble, Toronto, Ont. Inwood Brand Butter, The Inwood Creamery Co., Inwood, Man.

[3] Sunflower Oil supplied courtesy of CSP Foods, Winnipeg, Man.; Olive Oil, CS Gallo.

[4] Canola Oil supplied courtesy of CSP Foods, Winnipeg, Man.

[5] Soybean Oil supplied courtesy of Canada Packers Ltd., Toronto, Ont.

[6] Flax Oil, Omega Nutrition Inc., Vancouver, B.C.

Table 5. Daily Distribution of Added Fat in Different Diets

Food Item	Diet				
	Mixed Fat[1] (g)	S/O (g)	CAN (g)	SOY (g)	S/O/F[2] (g)
MENU I[3]					
Granola	12	12	12	12	12
Salad Dressing	5	5	5	5	6
Rice	10	10	10	10	9
Mashed Potatoes	24	26	26	26	22
Carrots	6	6	6	6	5
Cookies	24	24	24	24	24
Spread[4]	34	22	22	22	27
MENU II[3]					
Granola	12	12	12	12	12
Salad Dressing	5	5	5	5	6
Chili	21	21	21	21	18
Chicken Casserole	14	16	16	16	13
Noodles	5	5	5	5	5
Cookies	24	24	24	24	24
Spread[4]	34	22	22	22	27

[1] 5 g/day of corn oil was consumed as salad oil and 43 g/day of butter (34 g/day of butter fat) was consumed as spread. Lard, tallow, shortening, 23 g/day each, and 7 g of corn oil were blended to a uniform consistency and used in baking and preparing other food items shown above.

cont'd...

Table 5 (cont'd).

[2] 8 g/day of flax oil was added either to mashed potatoes or chili immediately prior to serving, and the remaining 27 g/day was made into a spread with butter. 36 g/day of sunflower oil was used in baked foods. An additional 13 g/day of sunflower oil was mixed with 21 g/day of olive oil and used in preparing other food items.

[3] Menu I and II of the two-day cyclic menu.

[4] All spread, except for the mixed fat diet, was made by blending the cooking oil or oil mixture with butter; 14 g/day of butter (11 g/day of butter fat) was used in the S/O, CAN and SOY diets, whereas 16 g/day of butter (13 g/day of butter fat) was used in the S/O/F diet.

Table 6. Saturated, Oleic, Linoleic and Linolenic Acids Composition of the Diets[1]

Diet	Fatty Acids (% of total)				Ratios	
	SFA	OA	LA	LNA	P/S[2]	LA/LNA
Mixed Fat	40.9	39.2	12.3	0.9	0.3	13.7
S/O	19.1	58.3	18.8	0.9	1.0	20.9
CAN	14.6	54.0	19.4	7.5	1.8	2.6
SOY	21.1	24.9	44.9	6.5	2.4	6.9
S/O/F	18.5	30.9	33.3	15.0	2.6	2.2

[1] Means of four duplicate diet samples
[2] P/S ratio = (LA + LNA) / SFA

experimental diets had about half the amount of saturated fatty acids present in the mixed fat diet. The S/O and CAN diets had similar amounts of OA and LA, but the CAN diet contained about eight times more LNA than the S/O diet. The SOY diet had a similar amount of LNA but had about twice the level of LA and about half as much OA as the CAN diet. The S/O/F diet had a similar LA/LNA ratio as the CAN diet, 2.2/1 and 2.6/1, respectively, but the LA and LNA levels were approximately twice those of the CAN diet. The amount of dietary cholesterol was about 100 mg higher in the mixed fat diet than in the other four diets as a consequence of the presence of more animal fat sources in this diet.

Meals were served in the metabolic unit in the Human Ecology Building situated at the University of Manitoba. Meals were served at customary times, but accommodated individual schedules as necessary. All food and beverages, including black coffee, clear tea, artificial sweetener and diet soft drinks were provided throughout the study. Emphasis was placed on the fact that no foods other than those provided were to be eaten.

Subjects weighed themselves daily before breakfast. Food consumption of the subjects was adjusted whenever persistent weight changes occurred. That is, body weight was monitored and food intakes were adjusted to maintain a constant body weight. When adjustments were necessary, special attention was taken to keep the fatty acid and macronutrient proportions

of the diet constant.

3.4 Handling, Preparation and Storage of Food Staples

All food was purchased as single lots from local suppliers, except for fresh produce, skim milk and bread, which were purchased biweekly from a single store. The fresh produce and skim milk were stored at 7°C in a conventional refrigerator whereas the bread was kept at -10°C until required for use. Dry staples, canned fruits and vegetables, and frozen juice and vegetables were stored under appropriate conditions. All fats and experimental oils were stored at 7°C in a walk-in refrigerator except for the flax oil, which was stored at -10°C.

Some food items were prepared about two months prior to the commencement of the study. Cookies and granola were baked and packaged. The ground beef for chili and the chicken breast for casseroles and sandwiches were cooked and portioned. Hamburger patties were portioned raw. All these portions were stored at -10°C in a walk-in freezer. Spread, except for the flax oil spread, was blended about one month prior to the start of the study and was stored at 7°C. To prevent oxidation of the flax oil, the flax oil spread was blended weekly and was kept at -10°C.

With the exception of the above mentioned items, all menu items were prepared in the metabolic unit just before serving. All items were carefully weighed and prepared following the

instructions described in the recipes. The recipes were similar to those used by Chan (1990). However, some modifications were made to improve the preparation methods as well as the incorporation of a higher level of dietary fats as presented in Appendix 5.

3.5 Diet Analysis

One-day duplicates of both menus for each of the diets in the two replicates were collected, homogenized in a Waring blender and aliquots were taken for proximate analysis. The aliquots were lyophilized to determine the moisture content of the samples. Using the lyophilized samples, protein content of the diets was determined by a modified Kjeldahl method (AACC method 46-12, modified by Williams, 1973), and the fat content was determined by the method of Bligh and Dyer (1959). The CHO content was derived by taking the difference between 100% and the sum of protein and fat content of the diets. The fatty acid composition of the fat extracted by the method of Bligh and Dyer (1959) was determined by gas chromatography (GC). The fatty acids were methylated using sodium methoxide ($NaOCH_3$) in methanol (CH_3OH) (Bannon et al., 1985). The fatty acid methyl esters were analyzed with a Hewlett Packard 5890 Gas Chromatograph equipped with a Durabond-225 capillary column, 30m x 0.25 mm, film thickness 0.25 microns (J & W Scientific Inc.). Injector, detector and column (oven) temperatures were: 250°C, 250°C and 205°C, respectively. The

carrier gas was helium set at a flow rate of 50 ml/min. Peak areas were measured with a Hewlett Packard 3392A integrator. The other GC conditions and integrator program are presented in Appendices 6 and 7, respectively. Fatty acid peaks were identified using a standard mix (cat. # GLC-68 B, Nu-Chek Prep. Inc., Elysian, MN). A sample of the standard mix chromatogram is included in Appendix 8. Also, a sample of the diet analysis chromatogram is presented in Appendix 9.

3.6 Plasma Lipid Analysis

3.6.1 Plasma Lipid and Lipoprotein Analysis

Blood samples were collected from each subject after a 12-hour overnight fast on Days 1, 7, 25, 43 and 49 of both replicates. Blood was collected from the antecubital vein using vacutainer tubes containing 0.10 ml of 15% EDTA (K_3) solution. Platelets were separated from the plasma samples by centrifuging the tubes at approximately 1,400 x g for 10-15 minutes. The platelet poor plasma was removed from these samples for analysis. Lipoprotein fractions in the plasma were separated by ultracentrifugation (Lindgren, 1975) for all samples collected. The VLDL fraction and HDL fraction were separated by centrifuging 0.6 ml plasma samples at a density of 1.0063 g/ml and a density of 1.0630 g/ml, respectively, for 18 hours at 104,000 x g. Plasma lipid and lipoprotein analysis was performed on fresh samples. Cholesterol was determined enzymatically in the plasma and lipoprotein

fractions by the method of Allain et al. (1974) and Bronzert and Brewer (1977) using a diagnostic kit (Fisher Scientific, Ottawa, Canada). Plasma TG also were analyzed using a diagnostic kit (Fisher Scientific, Ottawa, Canada) according to the enzymatic method by Fossatti and Lorenzo (1982) and McGowan et al. (1983). The rest of the platelet poor plasma was flushed with nitrogen and stored at -10°C until required for use in the PL and CE fatty acid analyses.

3.6.2 Modification of a Method for Extraction of Lipids from Plasma for PL and CE Fatty Acid Analysis

The main objectives for modifying the new method for extraction of lipids from plasma were: (i) it used smaller volumes of solvents, thus reducing environmental hazards and costs; and (ii) it used less glassware and had fewer steps, thus minimizing the possibility of contaminations, compared to the method by Folch et al. (1957). The new method for lipid extraction is similar to the method used in the Department of Foods and Nutrition, University of Manitoba (1990) for the lipid extraction of platelets. The main feature in this method was the change in the $CHCl_3$ / CH_3OH ratio from 2/1 (v/v) in the Folch et al. (1957) method, to 1/2 (v/v). However, just before the plasma samples were centrifuged, the ratio of $CHCl_3$ / CH_3OH was increased to 1/1 (v/v) by the addition of appropriate volumes of $CHCl_3$.

Using both the new and Folch et al. (1957) methods for

extraction of plasma lipid, the PL and CE fatty acid analyses were compared according to the procedures described in the next sections. The data generated by the two methods for lipid extraction is presented in the Results section. The new method for plasma lipid extraction was used in the CE fatty acid analysis, but not for the PL fatty acid analysis because of discrepancies in the results for the latter.

3.6.3 Plasma PL Fatty Acid Analysis

Lipids for PL fatty acid analysis were extracted from 1.0 ml of platelet poor plasma collected on Days 7, 25 and 43 using the method of Folch et al. (1957). Phospholipid fractions were separated using two-dimensional thin-layer chromatography (TLC). Each sample was spotted under nitrogen onto a heat-activated, pre-coated, 20x20 cm^2 silica gel 60 (without fluorescent indicator, 0.25 mm thick) plate (E. Merck) using a 5 μl Australia SGE syringe. Plates were run for about two hours in the first direction using $CHCl_3$ / CH_3OH / NH_4OH in a ratio of 65 / 35 / 5.5 (v/v/v) as the solvent system. The plates were dried under nitrogen for 30 minutes and exposed to HCl fumes for 10 minutes, to hydrolyse the ether linkages in the alkenylacyl ethanolamine phosphoglyceride (PPE). The plates were then dried for another 30 minutes before running in the second direction using the solvent system $CHCl_3$ / CH_3OH / formic acid in a ratio of 55 / 25 / 5 (v/v/v). After the second run of about

two hours, the developed plates were dipped in a dichlorofluorescein solution, exposed to ammonia fumes and observed under UV light. The various PL spots were identified using a standard plate supplied by the Department of Nutritional Sciences, University of Guelph. PC, PE and PPE spots were scraped from the plate and transferred to methylating tubes. Samples were methylated using $NaOCH_3$ in CH_3OH (Bannon et al., 1985) as described for Diet Analysis. The fatty acid methyl esters were again analyzed with a Hewlett Packard 5890 GC equipped with the same column and set at the same conditions as presented earlier for Diet Analysis. The integrator program was slightly different from the Diet Analysis (see Appendix 7). Fatty acid peaks were identified as described earlier. In addition, 20:5, 22:3 and 22:4 were identified using methylated standards for these fatty acids (Nu-Chek U-100-M, U-82-M and U-83-M, respectively). A sample of each standard chromatogram is included in Appendix 8. Pentadecaenoic acid (15:0) was used as an internal standard in all samples. A sample chromatogram for each of PC, PE and PPE is presented in Appendices 10, 11 and 12, respectively.

3.6.4 Plasma CE Fatty Acid Analysis

Lipids for CE fatty acid analysis were extracted from 0.2 ml platelet poor plasma of Days 7, 25 and 43 using the method for lipid extraction described in the previous section. The neutral lipids were separated using TLC. Before spotting

the samples, the TLC plates (same type as in PL analysis) were pre-washed overnight in a solvent system made up of petroleum ether / ethyl ether / glacial acetic acid in the ratio of 20 / 80 / 1 (v/v/v). Samples were spotted under nitrogen on the dried and heat-activated plates using the same type of syringe as described in PL analysis. The plates were developed in a solvent system of petroleum ether / ethyl ether / glacial acetic acid in a ratio of 80 / 20 / 1 (v/v/v) for 45 minutes. The developed plates were dipped in a dichlorofluorescein solution and observed under UV light. The CE was identified as the fastest migrating component of the seven lipid fractions separated; cholesteryl oleate (Sigma #C9253) was used to confirm this position. The CE spots were scraped and transferred to methylating tubes. Samples were methylated as described by Bannon et al. (1985). The fatty acid methyl esters were analyzed as described previously for the PL fractions. Again, 15:0 was used as an internal standard in all samples. The identification of fatty acid peaks was carried out as outlined for PL Analysis. A sample of the CE chromatogram is presented in Appendix 13.

3.6.5 Methylation and TLC Blanks

Methylation blanks were run regularly, one per set of methylations during the CE and PL analyses. In addition, a blank spot was scraped from every other TLC plate developed for CE analysis and from every TLC plate developed for PL

analysis. These blank spots were methylated and analyzed by the GC as TLC blanks. The amounts of fatty acids found in each sample were then adjusted for contamination present in the methylating and/or the TLC procedures (Appendix 14).

3.7 Statistical Analysis

For analysis of both the PL and CE method development data, means and standard deviations were calculated for each fatty acid.

A paired t-test was used to test for significant differences between the mixed fat diet and each experimental diet for all the data generated for lipids and lipoproteins, PL and CE. For the lipids and lipoproteins data, the above comparison was made as follows: Day 7 versus Day 25 and Day 43 versus Day 49. For the PL and CE, only Day 7 and Day 25 data was used for the above comparison.

Testing for the differences among the experimental diets was done using a one-way ANOVA model in PROC GLM of SAS (1984, 1986 SAS Institute Inc., Cary, N.C.) on the paired differences for each subject between Days 25 and 43 for the lipids and lipoproteins data. The PL and CE data on Days 25 and 43 were analyzed separately to test for differences among the experimental diets using a one-way ANOVA model in PROC GLM of SAS (1984, 1986 SAS Institute Inc., Cary, N.C.). The Fisher's protected least significant difference (LSD) approach was taken when testing for differences among the experimental

diets for lipids and lipoproteins, PL and CE data.

The above approach was taken with the assumption that there is no carry-over effect, since it is not relevant to test for any carry-over effect when the sample size is small. In other words, it will not be meaningful. This approach also took care of the imbalanced data due to the absence of one subject in Replicate II of the Experimental Design.

The SAS computer program (1984, 1986 SAS Institute Inc., Cary, N.C.) was utilized for all the above analyses. A representative sample of the SAS program used and the respective output generated are presented in Appendix 15.

4. RESULTS

4.1 Subjects

The absence of one subject in Replicate II was taken into account in the statistical analysis such that the approach taken was not affected by the missing values.

All subjects remained reasonably motivated throughout the study. Their compliance, which was monitored through recording weight changes and personal communication, was considered satisfactory. Variations in the weight of the subjects were in the range of ± 0.9 kg, except in five cases: subject 6 lost 1.2 kg; subjects 4 and 9 each lost 2.7 kg; subject 12 gained 2.4 kg; and subject one gained 2.9 kg.

4.2 Plasma Lipids and Lipoproteins

Figure 6 shows the patterns of change in mean plasma lipid levels in response to different diet sequences. Plotted values are means of the two replicates. Switching from the mixed fat diet to the experimental diets resulted in a significant ($p < 0.05$) decrease in the TC and LDL-C levels only in the CAN and SOY groups (Table 7). Switching the subjects to a different experimental diet at Day 25 did not exhibit any additional change in TC but some changes were observed in LDL-C levels. At the end of Experimental Period II, the mean difference in LDL-C levels of the CAN-S/O/F group was found to be significantly different from that of the SOY-CAN ($p < 0.003$) and S/O/F-S/O ($p < 0.02$) groups, but it

Figure 6. Changes in Mean Plasma Lipid Levels with Different Diet Sequences.

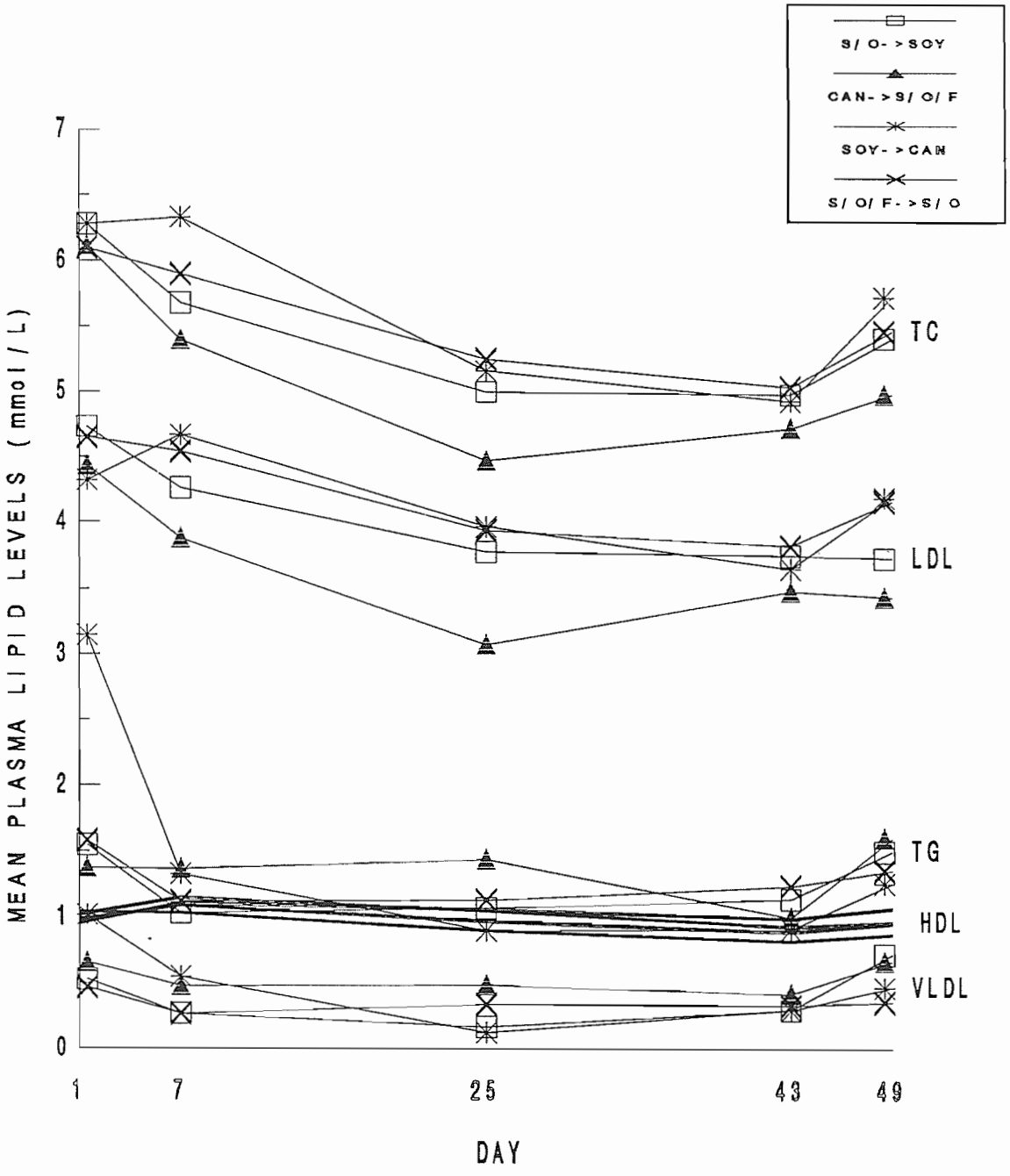


Table 7. Mean Plasma Lipid and Lipoprotein Levels Following the Mixed Fat and Experimental Fat Diets.[1]

Plasma Lipid	Diet (Day)			
	-- mmol/L --			
	MF[2] (7)	v. S/O (25)	SOY (43)	v. MF (49)
TC	5.7±0.4[a3]	5.0±0.1[a]	5.0±0.3[a]	5.4±0.6[a]
LDL-C	4.3±0.3[a]	3.8±0.2[a]	3.8±0.1[a]	3.7±0.6[a]
HDL-C	1.2±0.0[a]	1.1±0.0[b]	0.9±0.0[a]	1.0±0.0[a]
VLDL-C	0.3±0.1[a]	0.2±0.1[a]	0.3±0.2[a]	0.7±0.1[a]
TG	1.0±0.1[a]	1.1±0.1[a]	1.1±0.2[a]	1.5±0.2[b]
	MF (7)	v. SOY (25)	CAN (43)	v. MF (49)
TC	6.3±0.3[a]	5.2±0.3[b]	4.9±0.3[a]	5.7±0.3[b]
LDL-C	4.7±0.3[a]	4.0±0.3[b]	3.7±0.1[a]	4.2±0.2[b]
HDL-C	1.1±0.1[a]	1.1±0.1[a]	1.0±0.2[a]	1.1±0.1[a]
VLDL-C	0.6±0.2[a]	0.1±0.1[a]	0.3±0.2[a]	0.5±0.1[a]
TG	1.3±0.3[a]	0.9±0.2[a]	0.9±0.2[a]	1.3±0.3[a]
	MF (7)	v. CAN (25)	S/O/F (43)	v. MF (49)
TC	5.4±0.5[a]	4.5±0.4[b]	4.7±0.5[a]	5.0±0.5[a]
LDL-C	3.9±0.4[a]	3.1±0.3[b]	3.5±0.4[a]	3.4±0.4[a]
HDL-C	1.0±0.1[a]	0.9±0.0[a]	0.8±0.0[a]	0.9±0.1[a]
VLDL-C	0.5±0.2[a]	0.5±0.1[a]	0.4±0.0[a]	0.7±0.1[a]
TG	1.4±0.2[a]	1.4±0.2[a]	1.0±0.1[a]	1.6±0.3[a]

cont'd...

Table 7 (cont'd).

Plasma Lipid	Diet (Day)			
	-- mmol/L --			
	MF (7)	v. S/O/F (25)	S/O (43)	v. MF (49)
TC	5.9±0.5[a]	5.3±0.2[a]	5.0±0.3[a]	5.5±0.3[a]
LDL-C	4.5±0.5[a]	3.9±0.2[a]	3.8±0.3[a]	4.2±0.2[b]
HDL-C	1.1±0.0[a]	1.0±0.1[a]	0.9±0.1[a]	1.0±0.1[a]
VLDL-C	0.3±0.1[a]	0.3±0.1[a]	0.3±0.1[a]	0.4±0.1[a]
TG	1.1±0.3[a]	1.1±0.3[a]	1.2±0.2[a]	1.4±0.2[a]

[1] All values are mean ± SE. Individual subject data is presented in Appendix 16.
[2] Mixed Fat
[3] Values with the same superscript within each pair in the same row do not differ ($p>0.05$). T values obtained from the statistical analysis of diet means are shown in Appendix 21.

was not different from the S/O-SOY group (Table 8). This was due to an increase in the LDL-C level of the S/O/F group between Day 25 and Day 43 while the CAN and S/O groups showed a further drop in LDL-C levels, and the level for the SOY group remained constant. When all the subjects returned to the mixed fat diet during the post-experimental period, TC levels rose again, although the increase in TC was only significant in the CAN group ($p < 0.05$) (Table 7) while the LDL-C levels rose in two groups, viz., S/O and CAN groups ($p < 0.05$), and remained constant for the other two groups (Table 7). Nevertheless, the changes in TC levels were due primarily to the changes in LDL-C levels, although there were small but non-significant changes in VLDL-C levels. Changes in VLDL-C and TG followed more or less the same pattern described for TC and LDL-C, although the responses varied more among the experimental diets. There was no significant difference in the VLDL-C and TG levels among all the diets used in the study, except for (i) the rise in TG level in the SOY group when switched back to the mixed fat diet ($p < 0.05$) (Table 7), and (ii) the TG level of the S/O/F group was different from the other three groups, i.e. SOY ($p < 0.03$), CAN ($p < 0.04$) and S/O ($p < 0.02$), during Experimental Period II (Table 8). The latter difference was due to a drop in the TG level for the S/O/F group while the TG levels remained constant or increased slightly for the rest of the groups. Dietary fat source had no effect on the HDL-C levels except

Table 8. Mean Differences in Plasma Lipid Levels Between Days 25 and 43.

Diet Sequence	Plasma Lipids				
	TC	LDL-C	HDL-C	VLDL-C	TG
		---	mmol/L	---	
S/O -> SOY	0.0^{a1}	0.0^{ab}	0.1^{a}	-0.1^{a}	-0.1^{b2}
SOY -> CAN	0.2^{a}	0.3^{b3}	0.1^{a}	-0.2^{a}	0.0^{b4}
CAN -> S/O/F	-0.3^{a}	-0.4^{a}	0.1^{a}	0.1^{a}	0.4^{a}
S/O/F -> S/O	0.2^{a}	0.1^{b5}	0.1^{a}	0.0^{a}	-0.1^{b5}

[1] Values with the same superscript in the same column do not differ ($p > 0.05$).
[2] $p < 0.03$
[3] $p < 0.003$
[4] $p < 0.04$
[5] $p < 0.02$

for a slight drop in the S/O group when switched from the mixed fat to the S/O diet ($p < 0.05$) (Table 7). Differences observed could be attributable to diet change, day effect, or both, which makes it difficult to interpret the results.

4.3 Comparison of the Method of Folch et al. (1957) with a Modified Method for Extraction of Plasma Lipids

The fatty acid patterns of PL and CE extracted by a modification of the method used for the lipid extraction of platelets in the Department of Foods and Nutrition, University of Manitoba (1990) were compared with the patterns using the Folch et al. (1957) method for extracting the lipids.

4.3.1 Phospholipids

The fatty acid patterns of phospholipids extracted by the two methods are shown in Tables 9, 10, and 11 for PC, PE, and PPE, respectively. Large variations were observed for all the PL fractions using the modified method for plasma lipid extraction, as indicated by the standard deviations. No improvement was observed when the volume of solvent was doubled (Method II), i.e. standard deviations were still large. Thus, the method of Folch et al. (1957) was used to extract plasma lipids for the PL fatty acid analyses.

Table 9. Mean Plasma Phosphatidylcholine Fatty Acid Levels Using the Method of Folch et al. (1957) and a Modified Method for Lipid Extraction.[1]

Fatty Acid[2]	Method of Folch et al. (1957)	Method I	Method II[3]
	--- % of total fatty acid ---		
16:0	40.7± 1.6	38.6±26.5	35.2±31.4
16:1	1.0± 0.6	0.3± 0.4	3.6± 3.7
18:0	16.6± 0.3	10.3± 8.6	6.3± 6.4
18:1	12.7± 0.4	6.9± 6.3	4.1± 4.7
18:2	18.3± 0.9	10.2± 7.5	6.8± 6.4
18:3	0.9± 0.4	5.4± 7.5	6.2± 6.4
20:0	0.2± 0.2	0.1± 0.2	0.9± 0.9
20:1	0.1± 0.1	0.1± 0.1	0.0± 0.0
20:2	0.1± 0.1	0.1± 0.2	0.1± 0.2
20:3	1.7± 0.2	0.9± 0.9	0.4± 0.7
20:4	6.2± 0.8	3.9± 3.6	1.9± 2.3
20:5	0.4± 0.1	0.2± 0.3	1.1± 1.9
22:4	0.0± 0.0	0.0± 0.0	0.0± 0.0
22:5	0.0± 0.1	0.1± 0.2	0.0± 0.0
22:6	1.2± 0.2	0.7± 0.8	0.3± 0.5

[1] Values are mean ± SD
[2] Carbon chain length : number of double bonds
[3] Volume of solvent was doubled throughout

Table 10. Mean Plasma Phosphatidylethanolamine Fatty Acid Levels Using the Method of Folch et al. (1957) and a Modified Method for Lipid Extraction.[1]

Fatty Acid[2]	Method of Folch et al. (1957)	Method I	Method II[3]
	--- % of total fatty acid ---		
16:0	13.9± 0.5	12.3± 7.3	10.8± 9.4
16:1	0.6± 0.2	0.4± 0.2	0.7± 0.6
18:0	30.7± 0.3	25.4±14.6	21.4±18.6
18:1	13.4± 0.7	10.7± 6.1	9.2± 8.0
18:2	10.4± 0.4	7.2± 4.1	6.3± 5.4
18:3	0.3± 0.0	0.2± 0.2	0.2± 0.2
20:0	0.4± 0.1	0.3± 0.2	0.3± 0.2
20:1	0.4± 0.0	0.4± 0.2	0.3± 0.3
20:2	0.2± 0.0	0.1± 0.1	0.1± 0.1
20:3	0.9± 0.1	0.7± 0.4	0.6± 0.5
20:4	20.6± 0.5	14.3± 8.7	11.7±10.3
20:5	0.6± 0.1	0.4± 0.3	0.4± 0.3
22:4	0.6± 0.1	0.5± 0.3	0.4± 0.4
22:5	1.0± 0.1	0.7± 0.5	0.7± 0.6
22:6	6.1± 0.9	4.2± 2.6	3.8± 3.5

[1] Values are mean ± SD
[2] Carbon chain length : number of double bonds
[3] Volume of solvent was doubled throughout

Table 11. Mean Plasma Alkenylacyl Ethanolamine Phosphoglyceride Fatty Acid Levels Using the Method of Folch et al. (1957) and a Modified Method for Lipid Extraction.[1]

Fatty Acid[2]	Method of Folch et al. (1957)	Method I	Method II[3]
	--- % of total fatty acid ---		
16:0	0.3± 0.5	2.5± 5.2	0.0± 0.0
16:1	0.3± 0.3	0.0± 0.1	18.3±16.0
18:0	0.1± 0.1	0.6± 1.5	0.0± 0.0
18:1	2.7± 1.1	2.5± 1.7	0.0± 0.0
18:2	6.6± 1.1	6.7± 4.4	12.8±11.1
18:3	0.3± 0.1	2.8± 5.8	10.4± 9.0
20:0	0.0± 0.1	0.0± 0.0	0.0± 0.0
20:1	0.2± 0.3	0.1± 0.1	0.0± 0.0
20:2	0.0± 0.0	0.0± 0.0	0.0± 0.0
20:3	1.7± 0.1	1.1± 0.8	0.0± 0.0
20:4	58.4± 1.4	42.2±24.3	25.2±22.0
20:5	3.5± 0.2	2.3± 1.7	0.0± 0.0
22:4	4.8± 0.2	2.9± 2.3	0.0± 0.0
22:5	5.9± 0.4	3.3± 2.9	0.0± 0.0
22:6	15.3± 0.8	10.7± 6.6	0.0± 0.0

[1] Values are mean ± SD
[2] Carbon chain length : number of double bonds
[3] Volume of solvent was doubled throughout

4.3.2 Cholesteryl Esters

The results for the CE fatty acid analysis using the Folch et al. (1957) method and the modified method are shown in Table 12. Since the modified method and the Folch et al. (1957) method gave similar results, the modified method, which utilized less solvents and glassware, was chosen for the extraction of lipid from plasma for the CE fatty acid analysis.

4.4 Plasma PL and CE Fatty Acid Patterns

The plasma PC, PE, PPE and CE fatty acid data were subjected to the same statistical analysis. Since palmitic acid (PMA) and stearic acid (STEA) were hardly detectable in the PPE, they are not reported herein. Similarly docosatetraenoic acid (DTA) and docosapentaenoic acid (DPA) were rarely detectable in the CE, hence they were excluded from the CE data. PUFA of the n-6 and n-3 families with chain lengths longer than 18 carbons were combined in an attempt to see if the differences in the absolute and relative amounts of dietary LA and LNA had any effect on the overall levels of the desaturated and elongated PUFA in these families.

4.4.1 Comparison Between the Mixed Fat and the Experimental Fat Diets

Comparisons between the mixed fat diet and each experimental diet for each fatty acid in plasma PL and CE were

Table 12. Mean Plasma Cholesteryl Ester Fatty Acid Levels Using the Method of Folch et al. (1957) and a Modified Method for Lipid Extraction.[1]

Fatty Acid[2]	Method of Folch et al. (1957)	Modified Method
	--- % of total fatty acid ---	
16:0	12.3 ± 0.5	12.3 ± 0.4
16:1	2.7 ± 0.1	2.9 ± 0.1
18:0	1.5 ± 0.1	1.4 ± 0.4
18:1	20.3 ± 0.3	20.4 ± 0.1
18:2	51.6 ± 0.3	51.9 ± 0.3
18:3	0.7 ± 0.0	0.7 ± 0.1
20:3	0.8 ± 0.1	0.7 ± 0.0
20:4	8.3 ± 0.1	8.2 ± 0.2
20:5	0.9 ± 0.0	0.9 ± 0.0
22:6	0.8 ± 0.0	0.7 ± 0.1

[1] Values are mean ± SD
[2] Carbon chain length : number of double bonds

made statistically. As with the results observed in plasma lipids and lipoproteins, any differences observed could be due to diet change, day effect, or both, hence caution must be exercised in interpreting the effects of diet on the fatty acid patterns in these fractions.

4.4.1.1 Phosphatidylcholine (PC) (Table 13)

When compared to the mixed fat diet, the S/O diet resulted in a significantly lower level of EPA, while no significant changes were observed in any other fatty acids. The CAN diet, on the other hand, resulted in significantly higher levels of LNA and EPA than the mixed fat diet; again, no differences were observed for any other fatty acids. When compared with the mixed fat diet the SOY diet resulted in significantly lower levels of OA, eicosatrienoic acid (ETA), EPA, DTA, and total n-6 PUFA and significantly higher levels of LA and LNA. The S/O/F diet produced significantly lower levels of OA and total n-6 PUFA and significantly higher levels of LNA than the mixed fat diet. EPA also was higher for the S/O/F diet.

4.4.1.2 Phosphatidylethanolamine (PE) (Table 14)

In this fraction, the S/O diet resulted in significantly lower levels of STEA, LA, LNA, EPA and DPA and a significantly higher level of OA than the mixed fat diet. As with the PC, the CAN diet was associated with significantly higher levels

Table 13. Mean Plasma Phosphatidylcholine Fatty Acid Levels Following the Mixed Fat and the Experimental Diets.[1]

Fatty Acid(s)[2]	Diet (Day)			
	--- % of total fatty acids ---			
	MF[3] (7) v. S/O (25)		MF (7) v. CAN (25)	
16:0	36.9±2.3[a4]	35.8±4.5[a]	32.7±1.9[a]	29.1±1.8[a]
18:0	13.9±0.9[a]	15.3±2.4[a]	13.3±0.5[a]	13.5±0.6[a]
18:1	13.3±0.5[a]	14.1±1.6[a]	13.9±0.5[a]	16.1±0.9[a]
18:2	23.1±0.4[a]	19.8±2.9[a]	26.1±0.6[a]	25.5±0.8[a]
18:3	0.1±0.0[a]	0.1±0.0[a]	0.1±0.0[a]	0.4±0.1[b]
20:3	2.9±0.2[a]	3.0±0.3[a]	3.1±0.5[a]	2.6±0.3[a]
20:4	6.9±1.0[a]	8.3±1.7[a]	7.3±0.6[a]	7.9±1.0[a]
20:5	0.4±0.0[a]	0.2±0.0[b]	0.4±0.0[a]	1.0±0.2[b]
22:4	0.2±0.1[a]	0.2±0.1[a]	0.2±0.0[a]	0.2±0.0[a]
22:5	0.4±0.1[a]	0.3±0.1[a]	0.6±0.1[a]	0.7±0.1[a]
22:6	1.1±0.2[a]	1.5±0.4[a]	1.5±0.3[a]	1.8±0.4[a]
n-6[5]	10.0±1.2[a]	11.5±2.0[a]	10.6±0.7[a]	10.6±0.9[a]
n-3[6]	1.8±0.3[a]	2.0±0.6[a]	2.5±0.4[a]	3.4±0.7[a]

cont'd...

Table 13 (cont'd).

Fatty Acid(s)[2]	Diet (Day)			
	--- % of total fatty acids ---			
	MF[3] (7) v. SOY (25)		MF (7) v. S/O/F (25)	
16:0	29.7±1.4[a4]	32.9±0.7[a]	33.0±1.8[a]	34.4±2.3[a]
18:0	13.8±0.4[a]	14.1±0.7[a]	15.0±1.4[a]	16.3±1.2[a]
18:1	14.6±0.3[a]	8.4±0.6[b]	13.3±0.6[a]	9.9±0.6[b]
18:2	26.1±1.3[a]	32.0±1.2[b]	24.1±1.6[a]	26.0±1.9[a]
18:3	0.1±0.0[a]	0.3±0.0[b]	0.1±0.0[a]	0.8±0.1[b]
20:3	3.3±0.4[a]	1.6±0.2[b]	2.6±0.5[a]	1.5±0.2[a]
20:4	8.3±0.7[a]	7.9±0.9[a]	7.5±1.1[a]	6.4±1.1[a]
20:5	0.5±0.0[a]	0.3±0.0[b]	0.4±0.1[a]	0.7±0.2[b]
22:4	0.3±0.1[a]	0.1±0.0[b]	0.2±0.1[a]	0.2±0.1[a]
22:5	0.8±0.1[a]	0.5±0.1[a]	0.5±0.1[a]	0.6±0.1[a]
22:6	1.6±0.2[a]	1.1±0.1[a]	2.1±0.7[a]	1.8±0.3[a]
n-6[5]	11.9±1.1[a]	9.6±1.0[b]	10.3±1.6[a]	8.1±1.2[b]
n-3[6]	2.8±0.4[a]	1.9±0.2[a]	3.0±0.8[a]	3.1±0.4[a]

[1] All values are mean ± SE. Individual subject data is presented in Appendix 17.
[2] Carbon chain length : number of double bonds
[3] Mixed Fat
[4] Values with the same superscript within each pair in the same row do not differ ($p > 0.05$). T values obtained from statistical analysis of diet means are shown in Appendix 22.
[5] n-6 = 20:3 + 20:4 + 22:4
[6] n-3 = 20:5 + 22:5 + 22:6

Table 14. Mean Plasma Phosphatidylethanolamine Fatty Acid Levels Following the Mixed Fat and the Experimental Diets.[1]

Fatty Acid(s)[2]	Diet (Day)			
	--- % of total fatty acids ---			
	MF[3] (7) v.	S/O (25)	MF (7) v.	CAN (25)
16:0	17.6±2.5[a4]	15.7±2.4[a]	16.3±1.0[a]	13.7±1.0[a]
18:0	30.5±0.8[a]	29.8±0.9[b]	30.2±1.1[a]	30.4±1.2[a]
18:1	12.2±0.3[a]	16.2±1.0[b]	11.6±0.4[a]	14.9±1.5[a]
18:2	11.3±1.4[a]	10.6±1.3[b]	13.2±0.6[a]	11.9±1.4[a]
18:3	0.2±0.0[a]	0.1±0.0[b]	0.2±0.0[a]	0.5±0.1[b]
20:3	1.3±0.1[a]	1.5±0.2[a]	1.3±0.2[a]	1.3±0.2[a]
20:4	17.2±1.7[a]	17.8±2.3[a]	17.8±0.4[a]	16.6±1.1[a]
20:5	0.6±0.1[a]	0.3±0.1[b]	0.5±0.0[a]	1.1±0.2[b]
22:4	0.6±0.1[a]	0.6±0.2[a]	0.5±0.1[a]	0.2±0.1[b]
22:5	1.3±0.1[a]	0.8±0.1[b]	1.3±0.1[a]	1.3±0.1[a]
22:6	6.3±1.6[a]	5.5±1.5[a]	6.4±1.3[a]	6.9±1.8[a]
n-6[5]	19.1±1.8[a]	19.9±2.5[a]	19.6±0.5[a]	18.1±0.9[a]
n-3[6]	8.1±1.7[a]	6.6±1.6[a]	8.2±1.3[a]	9.2±1.9[a]

cont'd...

Table 14 (cont'd).

Fatty Acid(s)[2]	Diet (Day)			
	--- % of total fatty acids ---			
	MF[3] (7) v. SOY (25)		MF (7) v. S/O/F (25)	
16:0	17.5±3.0[a4]	13.2±1.6[a]	17.9±2.2[a]	13.8±1.6[b]
18:0	30.2±1.6[a]	31.9±1.8[a]	32.1±0.4[a]	33.2±0.9[a]
18:1	12.8±0.7[a]	10.8±0.6[a]	11.4±1.2[a]	11.6±0.9[a]
18:2	12.8±1.4[a]	16.1±1.7[b]	12.2±0.7[a]	15.8±0.9[a]
18:3	0.2±0.0[a]	0.4±0.1[b]	0.1±0.0[a]	0.9±0.0[b]
20:3	1.2±0.2[a]	0.9±0.1[a]	1.0±0.1[a]	0.8±0.1[a]
20:4	17.7±1.5[a]	18.3±0.7[a]	17.2±0.9[a]	15.7±0.4[a]
20:5	0.5±0.1[a]	0.4±0.0[a]	0.5±0.1[a]	0.8±0.1[b]
22:4	0.5±0.2[a]	0.4±0.0[a]	0.5±0.1[a]	0.3±0.1[b]
22:5	1.5±0.5[a]	1.4±0.1[a]	1.0±0.1[a]	1.3±0.2[a]
22:6	4.4±1.1[a]	4.9±0.8[a]	5.3±1.3[a]	4.7±0.9[a]
n-6[5]	19.3±1.8[a]	19.6±0.8[a]	18.6±1.0[a]	16.8±0.3[a]
n-3[6]	6.3±1.4[a]	6.8±0.8[a]	6.8±1.3[a]	6.7±0.7[a]

[1] All values are mean ± SE. Individual subject data is presented in Appendix 18.
[2] Carbon chain length : number of double bonds
[3] Mixed Fat
[4] Values with the same superscript within each pair in the same row do not differ ($p > 0.05$). T values obtained from statistical analysis of diet means are shown in Appendix 23.
[5] n-6 = 20:3 + 20:4 + 22:4
[6] n-3 = 20:5 + 22:5 + 22:6

of LNA and EPA. It also resulted in a significantly lower level of DTA than the mixed fat diet. As with the PC fraction, the SOY diet produced significantly higher levels of LA and LNA than the mixed fat diet, but there were no significant changes in any other fatty acids. As observed in the PC fraction, the S/O/F diet resulted in significantly higher levels of LNA and EPA than the mixed fat diet, but unlike the PC fraction the levels of PMA and DTA were significantly lower.

4.4.1.3 Alkenylacyl Ethanolamine Phosphoglyceride (PPE) (Table 15)

As observed in PC and PE fractions, the S/O diet was associated with significantly lower level of EPA than the mixed fat diet. The CAN diet resulted in a significantly higher level of LNA and significantly lower levels of ETA, DTA, total n-6 PUFA than the mixed fat diet. Surprisingly, no change was observed in EPA levels when the subjects were changed from the mixed fat diet to the CAN diet. As with the PC fraction, the SOY diet resulted in a significantly higher level of LA and significantly lower levels of ETA, EPA and DPA. It is rather surprising that the LNA level was not significantly higher following the SOY diet than following the mixed fat diet as observed in PC and PE. The S/O/F diet resulted in a significantly higher level of LNA, but unlike the PC and PE, not EPA, and a significantly lower level of

Table 15. Mean Plasma Alkenylacyl Ethanolamine Phosphoglyceride Fatty Acid Levels Following the Mixed Fat and the Experimental Diets.[1]

Fatty Acid(s)[2]	Diet (Day)			
	--- % of total fatty acids ---			
	MF[3] (7) v. S/O (25)		MF (7) v. CAN (25)	
18:1	4.9±0.7[a]	7.6±1.3[a]	5.1±0.8[a]	6.5±0.8[a]
18:2	13.7±2.7[a]	15.4±3.0[a]	16.4±1.8[a]	16.3±1.7[a]
18:3	0.3±0.2[a]	0.2±0.1[a]	0.3±0.1[a]	0.9±0.2[b]
20:3	2.4±0.3[a]	2.3±0.3[a]	2.7±0.5[a]	1.7±0.6[b]
20:4	47.6±4.5[a]	50.4±4.2[a]	49.5±0.7[a]	49.1±1.6[a]
20:5	2.9±0.3[a]	1.6±0.2[b]	3.1±0.1[a]	4.6±0.7[a]
22:4	2.0±0.1[a]	2.0±0.4[a]	2.2±0.3[a]	1.1±0.4[b]
22:5	4.7±0.5[a]	3.0±0.4[a]	5.6±0.6[a]	4.8±0.9[a]
22:6	15.0±2.2[a]	12.6±2.8[a]	14.1±2.9[a]	13.8±2.3[a]
n-6[5]	52.1±4.3[a]	54.8±4.1[a]	54.5±0.7[a]	51.8±1.1[b]
n-3[6]	22.6±1.9[a]	17.3±3.0[a]	22.8±2.3[a]	23.2±2.7[a]

cont'd...

Table 15 (cont'd).

Fatty Acid(s)[2]	Diet (Day)			
	--- % of total fatty acids ---			
	MF[3] (7) v. SOY (25)		MF (7) v. S/O/F (25)	
18:1	5.1±1.3[a]	3.7±0.7[a]	5.0±0.3[a]	3.8±0.5[a]
18:2	18.5±4.7[a]	25.7±3.8[b]	16.5±1.7[a]	18.4±1.2[a]
18:3	0.3±0.0[a]	0.8±0.2[a]	0.2±0.0[a]	1.1±0.0[b]
20:3	2.8±0.4[a]	1.2±0.1[b]	2.2±0.2[a]	1.2±0.1[b]
20:4	50.2±2.7[a]	49.5±2.9[a]	48.0±2.2[a]	46.9±2.3[a]
20:5	3.3±0.1[a]	1.9±0.1[b]	3.0±0.3[a]	4.0±0.6[a]
22:4	2.1±0.6[a]	1.5±0.5[a]	2.5±0.6[a]	2.1±0.1[a]
22:5	5.6±1.4[a]	4.0±1.0[b]	5.3±0.8[a]	5.8±0.5[a]
22:6	10.9±2.7[a]	10.4±3.0[a]	14.9±2.2[a]	15.1±2.4[a]
n-6[5]	55.0±3.3[a]	52.2±2.9[a]	52.7±2.9[a]	50.2±2.3[a]
n-3[6]	19.8±4.0[a]	16.3±3.8[a]	23.2±1.6[a]	24.9±2.0[a]

[1] All values are mean ± SE. Individual subject data is presented in Appendix 19.
[2] Carbon chain length : number of double bonds
[3] Mixed Fat
[4] Values with the same superscript within each pair in the same row do not differ ($p > 0.05$). T values obtained from statistical analysis of diet means are shown in Appendix 24.
[5] n-6 = 20:3 + 20:4 + 22:4
[6] n-3 = 20:5 + 22:5 + 22:6

ETA when compared to the mixed fat diet.

4.4.1.4 Cholesteryl Esters (CE) (Table 16)

When compared with the mixed fat diet, the S/O diet resulted in a significantly higher level of OA and significantly lower levels of LA and LNA as observed in the PE fraction. The CAN diet was associated with significantly lower levels of PMA and STEA and significantly higher levels of LA, LNA, EPA and total n-3 PUFA when compared with the mixed fat diet. Significantly higher levels of LNA and EPA also were observed for the PC and PE fractions. Following the SOY diet, significantly lower levels of PMA, OA, and ETA and significantly higher levels of LA and LNA were observed than following the mixed fat diet. The significantly higher levels of LA and LNA also were observed in the PC and PE fractions. Interestingly, the same pattern of changes was observed following the S/O/F diet as following the SOY diet. That is, the S/O/F diet was associated with significantly lower levels of PMA, OA, and ETA and significantly higher levels of LA and LNA when compared to the mixed fat diet. The significantly higher level of LNA also was observed in all PL fractions, but the other changes were not consistently observed in the PL fractions. Surprisingly, EPA was not significantly higher as observed in PC and PE fractions following the S/O/F diet as compared to the mixed fat diet.

Table 16. Mean Plasma Cholesteryl Ester Fatty Acid Levels Following the Mixed Fat and the Experimental Diets.[1]

Fatty Acid(s)[2]	Diet (Day)			
	--- % of total fatty acids ---			
	MF[3] (7) v. S/O (25)		MF (7) v. CAN (25)	
16:0	11.4±0.3[a4]	10.5±0.3[a]	12.5±0.2[a]	10.4±0.2[b]
18:0	1.3±0.1[a]	1.1±0.1[a]	1.5±0.1[a]	1.1±0.2[b]
18:1	20.9±0.2[a]	24.0±0.6[b]	21.2±0.8[a]	22.3±1.1[a]
18:2	51.2±0.4[a]	49.9±0.4[b]	53.7±1.0[a]	54.5±0.9[b]
18:3	0.5±0.1[a]	0.4±0.0[b]	0.5±0.0[a]	1.2±0.1[b]
20:3	0.9±0.2[a]	0.9±0.1[a]	0.7±0.1[a]	0.7±0.2[a]
20:4	8.6±1.0[a]	9.1±0.7[a]	6.4±1.0[a]	6.6±1.2[a]
20:5	0.9±0.1[a]	0.7±0.2[a]	0.6±0.1[a]	1.0±0.1[b]
22:6	0.8±0.1[a]	0.8±0.1[a]	0.5±0.2[a]	0.6±0.2[a]
n-6[5]	9.4±1.0[a]	10.1±0.7[a]	7.1±1.0[a]	7.3±1.3[a]
n-3[6]	1.7±0.1[a]	1.5±0.3[a]	1.1±0.3[a]	1.6±0.3[b]

cont'd...

Table 16 (cont'd).

Fatty Acid(s)[2]	Diet (Day)			
	--- % of total fatty acids ---			
	MF[3] (7) v. SOY (25)		MF (7) v. S/O/F (25)	
16:0	11.9±0.6[a4]	10.0±0.2[b]	12.1±0.2[a]	9.5±0.4[b]
18:0	1.5±0.2[a]	1.2±0.1[a]	1.2±0.1[a]	0.9±0.1[a]
18:1	21.0±0.6[a]	11.9±0.5[b]	19.8±0.4[a]	14.4±1.3[b]
18:2	54.2±1.8[a]	66.3±1.4[b]	54.8±0.7[a]	63.7±2.1[b]
18:3	0.4±0.0[a]	1.0±0.1[b]	0.4±0.0[a]	2.4±0.2[b]
20:3	0.7±0.1[a]	0.4±0.1[b]	0.6±0.1[a]	0.4±0.0[b]
20:4	6.7±0.6[a]	7.2±0.7[a]	7.0±0.4[a]	6.0±0.8[a]
20:5	0.6±0.1[a]	0.5±0.1[a]	0.6±0.1[a]	0.9±0.2[a]
22:6	0.5±0.1[a]	0.5±0.1[a]	0.8±0.2[a]	0.6±0.1[a]
n-6[5]	7.5±0.6[a]	7.6±0.8[a]	7.7±0.4[a]	6.4±0.8[a]
n-3[6]	1.0±0.1[a]	1.0±0.2[a]	1.4±0.3[a]	1.5±0.3[a]

[1] All values are mean ± SE. Individual subject data is presented in Appendix 20.
[2] Carbon chain length : number of double bonds
[3] Mixed Fat
[4] Values with the same superscript within each pair in the same row do not differ ($p > 0.05$). T values obtained from statistical analysis of diet means are shown in Appendix 25.
[5] n-6 = 20:3 + 20:4
[6] n-3 = 20:5 + 22:6

4.4.2 Comparison Among Different Experimental Fat Sources

4.4.2.1 Phosphatidylcholine (PC) (Tables 17 & 18)

The levels of OA and ETA in the PC fraction were significantly higher following the S/O and CAN diets than following the SOY and S/O/F diets on Day 25. The LA level was significantly higher following the SOY diet than the other three diets, while the level following the CAN and S/O/F diets was higher than following the S/O diet. The LNA level also was lowest following the S/O but highest following the S/O/F diet. The EPA content of PC was found to be significantly higher following the CAN and S/O/F diets than following the S/O diet. The EPA level also was higher following the CAN diet than following the SOY diet. However, the EPA level following the SOY diet did not differ from those following the S/O and S/O/F diets.

The fatty acid levels in the PC fraction on Day 43 (Table 18) were similar to those on Day 25 except for LA. The pattern for EPA was similar to that observed on Day 25 following each of the experimental diets. Similarly, the pattern for OA was similar, although the level of OA was significantly higher following the S/O/F diet than following the SOY diet on Day 43. On the other hand, there were no differences in LA levels among the diets on Day 43, although significant differences were observed on Day 25. The levels of LNA were significantly higher following the CAN and S/O/F diets than following the S/O and SOY diets on Day 43, whereas

Table 17. Mean Plasma Phosphatidylcholine Fatty Acid Levels Following Different Experimental Diets (Day 25).[1]

Fatty Acid(s)[2]	Experimental Diets			
	S/O	CAN	SOY	S/O/F
	-- % of total fatty acids --			
16:0	35.8±4.5[a3]	29.1±1.8[a]	32.9±0.7[a]	34.4±2.3[a]
18:0	15.3±2.4[a]	13.5±0.6[a]	14.1±0.7[a]	16.3±1.2[a]
18:1	14.1±1.6[a]	16.1±0.9[a]	8.4±0.6[b]	9.9±0.6[b]
18:2	19.8±2.9[a]	25.5±0.8[b]	32.0±1.2[c]	26.0±1.9[b]
18:3	0.1±0.0[a]	0.4±0.1[b]	0.3±0.0[c]	0.8±0.1[d]
20:3	3.0±0.3[a]	2.6±0.3[a]	1.6±0.2[b]	1.5±0.2[b]
20:4	8.3±1.7[a]	7.9±1.0[a]	7.9±0.9[a]	6.4±1.1[a]
20:5	0.2±0.0[a]	1.0±0.2[b]	0.3±0.0[ac]	0.7±0.2[bc]
22:4	0.2±0.1[a]	0.2±0.0[a]	0.1±0.0[a]	0.2±0.1[a]
22:5	0.3±0.1[a]	0.7±0.1[a]	0.5±0.1[a]	0.6±0.1[a]
22:6	1.5±0.4[a]	1.8±0.4[a]	1.1±0.1[a]	1.8±0.3[a]
n-6[4]	11.5±2.0[a]	10.6±0.9[a]	9.6±1.0[a]	8.1±1.2[a]
n-3[5]	2.0±0.6[a]	3.4±0.7[a]	1.9±0.2[a]	3.1±0.4[a]

[1] All values are mean ± SE. Individual subject data is presented in Appendix 17.
[2] Carbon chain length : number of double bonds
[3] Values in the same row with the same superscript letter do not differ ($p > 0.05$). P values obtained from the statistical comparison of diet means are shown in Appendix 26.
[4] n-6 = 20:3 + 20:4 + 22:4
[5] n-3 = 20:5 + 22:5 + 22:6

Table 18. Mean Plasma Phosphatidylcholine Fatty Acid Levels Following Different Experimental Diets (Day 43).[1]

Fatty Acid(s)[2]	Experimental Diets			
	S/O	CAN	SOY	S/O/F
	-- % of total fatty acids --			
16:0	34.0±0.8[a3]	29.8±3.1[a]	34.2±0.7[a]	32.4±1.9[a]
18:0	12.6±0.4[a]	12.6±0.9[a]	14.7±0.7[a]	13.7±0.4[a]
18:1	15.7±0.7[a]	16.8±0.7[a]	8.3±0.6[b]	11.5±0.4[c]
18:2	25.6±1.5[a]	26.3±1.1[a]	28.9±0.8[a]	29.3±1.1[a]
18:3	0.1±0.0[a]	0.5±0.0[b]	0.3±0.0[a]	0.6±0.1[b]
20:3	2.4±0.0[a]	1.8±0.3[a]	2.3±0.4[a]	2.2±0.4[a]
20:4	7.0±1.1[a]	8.0±1.3[a]	8.1±0.2[a]	6.7±1.0[a]
20:5	0.2±0.0[a]	0.9±0.2[b]	0.4±0.1[ac]	0.7±0.1[bc]
22:4	0.1±0.0[a]	0.1±0.0[a]	0.1±0.0[a]	0.1±0.1[a]
22:5	0.3±0.0[a]	0.6±0.2[a]	0.5±0.1[a]	0.6±0.1[a]
22:6	1.0±0.2[a]	1.2±0.3[a]	1.2±0.1[a]	1.3±0.3[a]
n-6[4]	9.4±1.1[a]	9.9±1.7[a]	10.5±0.1[a]	9.1±1.1[a]
n-3[5]	1.5±0.2[a]	2.7±0.7[a]	2.2±0.1[a]	2.6±0.4[a]

[1] All values are mean ± SE. Individual subject data is presented in Appendix 17.
[2] Carbon chain length : number of double bonds
[3] Values in the same row with the same superscript letter do not differ ($p > 0.05$). P values obtained from the statistical comparison of diet means are shown in Appendix 27.
[4] n-6 = 20:3 + 20:4 + 22:4
[5] n-3 = 20:5 + 22:5 + 22:6

the level was highest following the S/O/F diet and lowest following the S/O diet on Day 25. Similarly, no significant differences were observed in the levels of ETA on Day 43, whereas differences were observed on Day 25.

4.4.2.2 Phosphatidylethanolamine (PE) (Tables 19 & 20)

Fatty acid levels in the PE fraction on Day 25 followed similar patterns to those of the PC fraction on Day 25, although there were notable differences. As for the PC fraction, the levels of OA were significantly higher following the S/O and CAN diets than following the SOY and S/O/F diets. Similarly, the LA level was significantly higher following the SOY diet than following the S/O and CAN diets but the level on the SOY diet did not differ from the level on the S/O/F diet. Similarly, the LNA level in plasma PE was similar to that of the PC fraction on Day 25 except that there was no difference between the CAN and SOY diets. A significantly higher level of ETA was observed following the S/O diet than following the SOY and S/O/F diets but the level following the CAN diet did not differ from that following the SOY diet. However, a significantly higher level of ETA was observed following the CAN than the S/O/F diet. As for the PC fraction, no differences were observed in the levels of AA, DTA, DHA, total n-6 and n-3 PUFA among the experimental diets. By contrast, the level of EPA was significantly higher following the CAN diet than the other three diets and there was no difference

Table 19. Mean Plasma Phosphatidylethanolamine Fatty Acid Levels Following Different Experimental Diets (Day 25).[1]

Fatty Acid(s)[2]	Experimental Diets			
	S/O	CAN	SOY	S/O/F
	-- % of total fatty acids --			
16:0	15.7±2.4[a3]	13.7±1.0[a]	13.2±1.6[a]	13.8±1.6[a]
18:0	29.8±0.9[a]	30.4±1.2[a]	31.9±1.8[a]	33.2±0.9[a]
18:1	16.2±1.0[a]	14.9±1.5[a]	10.8±0.6[b]	11.6±0.9[b]
18:2	10.6±1.3[a]	11.9±1.4[ac]	16.1±1.7[b]	15.8±0.9[bc]
18:3	0.1±0.0[a]	0.5±0.1[b]	0.4±0.1[b]	0.9±0.0[c]
20:3	1.5±0.2[a]	1.3±0.2[ac]	0.9±0.1[bc]	0.8±0.1[b]
20:4	17.8±2.3[a]	16.6±1.1[a]	18.3±0.7[a]	15.7±0.4[a]
20:5	0.3±0.1[a]	1.1±0.2[b]	0.4±0.0[a]	0.8±0.1[c]
22:4	0.6±0.2[a]	0.2±0.1[a]	0.4±0.0[a]	0.3±0.1[a]
22:5	0.8±0.1[a]	1.3±0.1[b]	1.4±0.1[b]	1.3±0.2[b]
22:6	5.5±1.5[a]	6.9±1.8[a]	4.9±0.8[a]	4.7±0.9[a]
n-6[4]	19.9±2.5[a]	18.1±0.9[a]	19.6±0.8[a]	16.8±0.3[a]
n-3[5]	6.6±1.6[a]	9.2±1.9[a]	6.8±0.8[a]	6.7±0.7[a]

[1] All values are mean ± SE. Individual subject data is presented in Appendix 18.
[2] Carbon chain length : number of double bonds
[3] Values in the same row with the same superscript letter do not differ ($p > 0.05$). P values obtained from the statistical comparison of diet means are shown in Appendix 28.
[4] n-6 = 20:3 + 20:4 + 22:4
[5] n-3 = 20:5 + 22:5 + 22:6

Table 20. Mean Plasma Phosphatidylethanolamine Fatty Acid Levels Following Different Experimental Diets (Day 43).[1]

Fatty Acid(s)[2]	Experimental Diets			
	S/O	CAN	SOY	S/O/F
	-- % of total fatty acids --			
16:0	15.2±1.9[a3]	13.5±1.0[a]	14.9±1.1[a]	14.2±1.7[a]
18:0	29.3±0.3[ad]	28.0±1.7[a]	33.6±1.2[bc]	32.0±1.2[cd]
18:1	16.6±1.4[a]	20.0±0.3[b]	9.8±0.6[c]	11.1±0.5[c]
18:2	14.0±1.1[a]	12.4±1.1[a]	14.0±1.0[a]	15.0±1.6[a]
18:3	0.1±0.0[a]	0.6±0.0[bc]	0.4±0.0[ac]	0.7±0.2[b]
20:3	1.3±0.1[a]	0.9±0.0[a]	1.1±0.1[a]	1.1±0.2[a]
20:4	16.5±1.2[a]	16.5±0.6[a]	17.4±0.6[a]	16.9±1.3[a]
20:5	0.3±0.0[a]	0.9±0.1[b]	0.4±0.1[a]	0.8±0.1[b]
22:4	0.4±0.1[a]	0.1±0.0[b]	0.5±0.1[a]	0.4±0.1[a]
22:5	0.8±0.1[a]	1.3±0.2[a]	1.2±0.2[a]	1.5±0.2[a]
22:6	4.4±1.2[a]	4.5±0.3[a]	5.6±0.7[a]	5.4±1.4[a]
n-6[4]	18.2±1.3[a]	17.4±0.6[a]	19.0±0.7[a]	18.3±1.3[a]
n-3[5]	5.4±1.2[a]	6.7±0.3[a]	7.2±1.0[a]	7.7±1.5[a]

[1] All values are mean ± SE. Individual subject data is presented in Appendix 18.
[2] Carbon chain length : number of double bonds
[3] Values in the same row with the same superscript letter do not differ ($p > 0.05$). P values obtained from the statistical comparison of diet means are shown in Appendix 29.
[4] n-6 = 20:3 + 20:4 + 22:4
[5] n-3 = 20:5 + 22:5 + 22:6

between the S/O and SOY diets. Similarly, DPA was found to be significantly lower following the S/O diet than the other three experimental diets.

Fatty acid patterns of the PE fraction on Day 43 (Table 20) were similar to those of the PC fraction on Day 43 except for STEA and DTA. However, there were more differences in the fatty acid pattern for the PE than PC fraction on Day 43 versus Day 25. STEA was significantly higher on the SOY and S/O/F than the CAN diet and the level of STEA also was significantly higher following the SOY than following the S/O diet. Similarly, the level of OA was significantly higher on the CAN than the S/O diet on Day 43 and no significant differences were observed in LA, ETA or DHA levels following the experimental diets. By contrast, some differences were observed in LNA levels, such that the LNA level was highest for the S/O/F diet, lowest for the S/O diet, and intermediate for the other two diets. As on Day 25, no significant differences among the diets were found for ETA, AA, DPA, DHA, total n-6 and n-3 PUFA. For EPA, both CAN and S/O/F reflected significantly higher levels than following either S/O or SOY diet. The level of DTA was significantly lower following the CAN diet than the other diets, although a similar trend was observed on Day 25.

4.4.2.3 Alkenylacyl Ethanolamine Phosphoglyceride (PPE) (Tables 21 & 22)

The fatty acid patterns of the PPE fraction on Day 25 (Table 21) were exactly the same as those of the PPE fraction on Day 43 (Table 22), except for the levels of LNA. The level of LNA on Day 43 was significantly higher for the S/O/F diet than the other three diets.

Not surprisingly, significantly higher levels of OA were observed following the S/O and CAN diets than following the SOY and S/O/F diets on both Days 25 and 43 for the PPE fraction. This observation is consistent with the findings for the PC and PE fractions. Likewise, there was no difference in the level of LA among the experimental diets on either Day 25 or 43, which is consistent with the patterns for PC and PE on Day 43. As with PC and PE, the level of LNA was significantly lower following the S/O diet than the other diets on Day 25, and the level of LNA usually was highest following the S/O/F diet. No significant differences in the levels of ETA, AA, DTA, DPA, DHA, total n-6 and n-3 PUFA were found among the experimental diets. These observations were the same as for the PC and PE fractions except for ETA and DPA (for PE only) on Day 25. The levels of EPA were found to be significantly higher following the CAN and S/O/F diets than following either the S/O or SOY diets, which is similar to the pattern for PE.

Table 21. Mean Plasma Alkenylacyl Ethanolamine Phosphoglyceride Fatty Acid Levels Following Different Experimental Diets (Day 25).[1]

Fatty Acid(s)[2]	Experimental Diets			
	S/O	CAN	SOY	S/O/F
	-- % of total fatty acids --			
18:1	7.6±1.3[a3]	6.5±0.8[a]	3.7±0.7[b]	3.8±0.5[b]
18:2	15.4±3.0[a]	16.3±1.7[a]	25.7±3.8[a]	18.4±1.2[a]
18:3	0.2±0.1[a]	0.9±0.2[b]	0.8±0.2[b]	1.1±0.0[b]
20:3	2.3±0.3[a]	1.7±0.6[a]	1.2±0.1[a]	1.2±0.1[a]
20:4	50.4±4.2[a]	49.1±1.6[a]	49.5±2.9[a]	46.9±2.3[a]
20:5	1.6±0.2[a]	4.6±0.7[b]	1.9±0.1[a]	4.0±0.6[b]
22:4	2.0±0.4[a]	1.1±0.4[a]	1.5±0.5[a]	2.1±0.1[a]
22:5	3.0±0.4[a]	4.8±0.9[a]	4.0±1.0[a]	5.8±0.5[a]
22:6	12.6±2.8[a]	13.8±2.3[a]	10.4±3.0[a]	15.1±2.4[a]
n-6[4]	54.8±4.1[a]	51.8±1.1[a]	52.2±2.9[a]	50.2±2.3[a]
n-3[5]	17.3±3.0[a]	23.2±2.7[a]	16.3±3.8[a]	24.9±2.0[a]

[1] All values are mean ± SE. Individual subject data is presented in Appendix 19.
[2] Carbon chain length : number of double bonds
[3] Values in the same row with the same superscript letter do not differ ($p > 0.05$). P values obtained from the statistical comparison of diet means are shown in Appendix 30.
[4] n-6 = 20:3 + 20:4 + 22:4
[5] n-3 = 20:5 + 22:5 + 22:6

Table 22. Mean Plasma Alkenylacyl Ethanolamine Phosphoglyceride Fatty Acid Levels Following Different Experimental Diets (Day 43).[1]

Fatty Acid(s)[2]	Experimental Diets S/O	CAN	SOY	S/O/F
	-- % of total fatty acids --			
18:1	7.0±0.8[a3]	7.4±1.1[a]	2.8±0.3[b]	4.4±0.9[b]
18:2	18.2±1.6[a]	18.7±3.1[a]	15.2±2.0[a]	20.0±2.4[a]
18:3	0.1±0.1[a]	0.7±0.1[b]	0.5±0.1[ab]	1.7±0.3[c]
20:3	1.9±0.2[a]	1.5±0.1[a]	1.4±0.2[a]	1.8±0.6[a]
20:4	48.9±2.3[a]	49.8±1.6[a]	51.4±3.9[a]	48.7±2.2[a]
20:5	1.7±0.1[a]	4.4±0.4[b]	2.1±0.3[a]	3.7±0.5[b]
22:4	1.9±0.3[a]	1.2±0.2[a]	2.4±0.8[a]	1.3±0.3[a]
22:5	3.6±0.6[a]	4.2±0.8[a]	5.7±2.1[a]	5.1±0.8[a]
22:6	13.6±3.2[a]	10.5±2.3[a]	16.9±2.2[a]	12.0±1.8[a]
n-6[4]	52.8±2.3[a]	52.4±1.6[a]	55.2±3.2[a]	51.8±1.8[a]
n-3[5]	18.8±3.4[a]	19.1±3.4[a]	24.7±4.4[a]	20.8±0.6[a]

[1] All values are mean ± SE. Individual subject data is presented in Appendix 19.
[2] Carbon chain length : number of double bonds
[3] Values in the same row with the same superscript letter do not differ ($p > 0.05$). P values obtained from the statistical comparison of diet means are shown in Appendix 31.
[4] n-6 = 20:3 + 20:4 + 22:4
[5] n-3 = 20:5 + 22:5 + 22:6

4.4.2.4 Cholesteryl Esters (CE) (Tables 23 & 24)

The levels of PMA and STEA were not significantly different among the experimental diets on either Day 25 or 43. This pattern is consistent with the observations for the PC fraction on Days 25 and 43 and the PE fraction on Day 25. OA was significantly higher following the S/O and CAN diets than following the SOY and S/O/F diets. By contrast, LA was significantly higher following the SOY and S/O/F diets. The LNA level was significantly higher following the CAN, SOY and S/O/F diets than the S/O diet with the level on the S/O/F diet being appreciably higher than on the CAN and SOY diets. No differences were observed between the CAN and SOY diets for this fatty acid. The LNA pattern was similar to that of PC and PE on Day 25. The ETA level on Day 25 was found to be significantly higher following the S/O diet than following the SOY and S/O/F diets, while there was no significant difference between the S/O and CAN diets, which is the same pattern as for PC and PE on Day 25. Diet had no effect on the EPA level on Day 25, but the level following the CAN diet was significantly higher than following the S/O diet on Day 43. The experimental diets had no impact on the levels of AA, DHA, total n-6 and n-3 PUFA.

4.5 Summary

A significant reduction in plasma TC and LDL-C levels was observed in the CAN and SOY groups at the end of Experimental

Table 23. Mean Plasma Cholesteryl Ester Fatty Acid Levels Following Different Experimental Diets (Day 25).[1]

Fatty Acid(s)[2]	Experimental Diets			
	S/O	CAN	SOY	S/O/F
	-- % of total fatty acids --			
16:0	10.6±0.3[a3]	10.4±0.2[a]	10.0±0.1[a]	9.5±0.4[a]
18:0	1.1±0.1[a]	1.1±0.2[a]	1.2±0.1[a]	0.9±0.1[a]
18:1	24.0±0.5[a]	22.3±1.1[a]	11.9±0.5[b]	14.4±1.3[b]
18:2	49.9±0.4[a]	54.5±0.9[a]	66.3±1.4[b]	63.7±2.1[b]
18:3	0.4±0.0[a]	1.2±0.1[b]	1.0±0.1[b]	2.4±0.2[c]
20:3	0.9±0.1[a]	0.7±0.2[ab]	0.4±0.1[b]	0.4±0.0[b]
20:4	9.1±0.7[a]	6.6±1.2[a]	7.2±0.7[a]	6.0±0.8[a]
20:5	0.7±0.2[a]	1.0±0.1[a]	0.5±0.1[a]	0.9±0.2[a]
22:6	0.8±0.1[a]	0.6±0.2[a]	0.5±0.1[a]	0.6±0.1[a]
n-6[4]	10.1±0.7[a]	7.3±1.3[a]	7.6±0.8[a]	6.4±0.8[a]
n-3[5]	1.5±0.3[a]	1.5±0.3[a]	1.0±0.2[a]	1.5±0.2[a]

[1] All values are mean ± SE. Individual subject data is presented in Appendix 20.
[2] Carbon chain length : number of double bonds
[3] Values in the same row with the same superscript letter do not differ ($p > 0.05$). P values obtained from the statistical comparison of diet means are shown in Appendix 32.
[4] n-6 = 20:3 + 20:4
[5] n-3 = 20:5 + 22:6

Table 24. Mean Plasma Cholesteryl Ester Fatty Acid Levels Following Different Experimental Diets (Day 43).[1]

Fatty Acid(s)[2]	Experimental Diets			
	S/O	CAN	SOY	S/O/F
	-- % of total fatty acids --			
16:0	10.3±0.3[a3]	10.0±0.4[a]	10.0±0.6[a]	10.7±0.1[a]
18:0	0.8±0.0[a]	0.9±0.1[a]	1.0±0.1[a]	1.2±0.1[a]
18:1	22.0±0.7[a]	20.9±0.6[a]	11.7±0.8[b]	17.3±0.4[c]
18:2	56.0±1.7[a]	56.7±1.4[a]	64.3±1.1[b]	59.8±0.9[a]
18:3	0.3±0.0[a]	1.3±0.1[bc]	0.9±0.0[b]	1.9±0.3[c]
20:3	0.6±0.0[a]	0.4±0.0[a]	0.6±0.8[a]	0.5±0.1[a]
20:4	7.3±1.2[a]	6.9±0.7[a]	8.4±0.8[a]	5.7±1.0[a]
20:5	0.4±0.0[a]	1.0±0.2[b]	0.7±0.1[ab]	0.7±0.1[ab]
22:6	0.6±0.1[a]	0.5±0.1[a]	0.8±0.1[a]	0.5±0.2[a]
n-6[4]	7.9±1.2[a]	7.3±0.7[a]	9.1±0.8[a]	6.2±1.0[a]
n-3[5]	1.0±0.05[a]	1.6±0.2[a]	1.4±0.1[a]	1.2±0.3[a]

[1] All values are mean ± SE. Individual subject data is presented in Appendix 20.
[2] Carbon chain length : number of double bonds
[3] Values in the same row with the same superscript letter do not differ ($p > 0.05$). P values obtained from the statistical comparison of diet means are shown in Appendix 33.
[4] n-6 = 20:3 + 20:4
[5] n-3 = 20:5 + 22:6

Period I. Changing the subjects to a different experimental fat at Day 25 did not produce any further change in TC. However, changes were observed in LDL-C levels. There was an increase in the LDL-C level of the S/O/F group, while the level for the CAN and S/O groups decreased further. The level for the SOY group did not change during Experimental Period II. Experimental diets had no effect on HDL-C and VLDL-C levels.

Fatty acid patterns of plasma PL changed appreciably in response to changes in diets. The PMA and STEA levels were appreciably higher on the mixed fat than the experimental diets. When subjects were switched from the mixed fat diet to the experimental diets, changes in OA, LA and LNA levels in the plasma PL and CE reflected the fatty acid composition of the diets fairly closely. Generally, the following PL and CE fatty acid patterns were observed with the mixed fat and experimental diets: the S/O diet was associated with significantly higher level of OA and significantly lower levels of LNA and EPA than the mixed fat diet; the CAN diet was associated with significantly higher levels of LNA and EPA than the mixed fat diet; the SOY diet was associated with significantly lower levels of OA, ETA and EPA and significantly higher levels of LA and LNA than the mixed fat diet; the S/O/F diet was associated with lower levels of PMA, OA and ETA and higher levels of LNA and EPA than the mixed fat diet. In general, these differences reflected the differences

in the fatty acid composition of the diets.

Likewise, differences in fatty acid composition of the PL and CE fractions among the experimental diets reflected differences in the fatty acid levels of the diets. For fatty acids with chain length shorter than 20 carbons, fatty acid levels of the plasma lipids following the experimental diets reflected closely the fatty acid composition of the diets. Diets which had high levels of OA (the S/O and CAN diets), LA (the SOY and S/O/F diets) and LNA (the CAN, SOY and S/O/F diets) were associated with significantly higher levels of OA, LA, LNA, respectively, in plasma PL and CE. The SOY and S/O/F diets were usually associated with significantly lower levels of ETA in plasma PL and CE on Day 25 but not on Day 43 than the S/O and CAN diets. AA in plasma lipids was particularly stable. There were no significant changes in AA level in plasma lipids following any of the experimental diets on either Day 25 or 43. Both the CAN and S/O/F diets were frequently associated with significantly higher levels of EPA in plasma lipids on Days 25 and 43. The experimental diets did not seem to affect the levels of DTA, DPA, DHA, total n-6 and n-3 PUFA in plasma lipids at the end of either experimental period.

5. DISCUSSION

5.1 Plasma Lipid and Lipoprotein Patterns

The mixed fat diet had about twice the amount of SFA as the experimental diets, which varied in the amounts of OA, LA and LNA. Hence, it is not surprising to see that plasma cholesterol levels were higher following the mixed fat diet than following the experimental fat diets. The hypercholesterolemic effect of SFA has been proven consistently (Grundy & Denke, 1990; Hegsted et al., 1965; Keys, 1970; Keys et al., 1957; Shepherd et al., 1978, 1980; Spady & Dietschy, 1988; Vega et al., 1982). A significant reduction ($p < 0.05$) in mean plasma TC and LDL-C levels was observed in the CAN and SOY groups at the end of Experimental Period I. Although the decrease in mean plasma TC and LDL-C levels for the S/O and S/O/F groups during Experimental Period I was not significant, a similar pattern was observed as for the CAN and SOY groups. The changes in TC levels were due primarily to the changes in LDL-C levels, although there were small but non-significant changes in VLDL-C levels. The cholesterol-lowering effect of OA and LA also has been clearly demonstrated (Baggio et al., 1988; Chan, 1990; Cortese et al., 1983; Mattson & Grundy, 1985; Masana et al., 1991; McDonald et al., 1989; Mensink & Katan, 1987, 1989; Shepherd et al., 1978; Vega et al., 1982; Wardlaw & Snook, 1990) except in earlier studies where the hypocholesterolemic effect of OA was not observed (Hegsted et al., 1965; Keys et al., 1957, 1965a).

However, the results of the earlier studies may be questioned since changes in OA consumption were accompanied by variations in total fat intake (Keys et al., 1965a) or SFA level of the diets (Keys et al., 1957). Furthermore, OA levels in the diets were not always tested (Hegsted et al., 1965; Keys et al., 1957). Hence, the failure to see the hypocholesterolemic effect of OA in earlier studies could be attributed to the lack of proper control over the levels of fatty acids and total fat in the diets. Keys et al. (1965a) and Lasserre et al. (1985) observed that an OA-rich diet was as effective as LA-rich or low-fat diets in lowering cholesterol, although only a modest hypocholesterolemic effect was observed by Renaud et al. (1986) when canola and/or sunflower oil replaced saturated fat in the diets.

The hypocholesterolemic effect of LNA is not as clearly demonstrated as that of OA and LA. Inconsistent changes in plasma cholesterol levels were reported by Sanders and Roshanai (1983) and Mest et al. (1983), where subjects were given linseed oil supplements, which were high in LNA, although these results may have been compromised by a failure to control dietary fat content in these studies. Chan (1990), on the other hand, found that dietary OA, LA and LNA had similar cholesterol-lowering effects in a group of normal healthy subjects fed diets in which levels of fat were carefully controlled. In the present study, the hypocholesterolemic effects of OA, LA and LNA were not as

clearly demonstrated in a group of hyperlipidemic men as reported by Chan (1990) with normolipidemic individuals. This difference was mainly due to the large variation in the responses among individuals in the present study.

Mattson and Grundy (1985) found that OA and LA had similar hypocholesterolemic effects in a group of hypercholesterolemic subjects. However, there was considerable variability among subjects in response both to the high OA and high LA diets. Grundy and Vega (1988) studying the variability in responsiveness to SFA found that some hyperlipidemic patients demonstrated a striking rise in cholesterol levels, whereas others had more modest increases. They stated that the variability in responsiveness to SFA, i.e. either hyperresponders or poor responders, were genetically determined, although the mechanisms responsible for these extreme differences remain debatable. Keys et al. (1959, 1965b) also examined inherent differences in individual responses to different kinds of fatty acids in the diet. They found that individuals having the highest cholesterol levels following a diet high in SFA tended to have the greatest reductions in cholesterol concentrations following a diet high in unsaturated fatty acids.

The mixed fat diet had about 100 mg/day more cholesterol than the experimental diets due to the presence of more animal fats in the mixed fat diet. However, this should not be of great concern since Grundy (1986) and Hegsted (1965) found

that dietary cholesterol had an insignificant effect on plasma TC levels. For instance, a decrease of 100 mg/day of cholesterol caused TC level to drop by only 0.1 mmol/L.

The two experimental diets which were found to have similar cholesterol-lowering effects, viz. CAN and SOY, had different unsaturated fatty acid composition. The CAN diet had about twice as much OA and half as much LA as the SOY diet; the LNA content was essentially the same for these diets. Although the results of the present study do not give direct evidence whether OA, LA and LNA act via similar mechanisms in affecting lipoprotein metabolism, it is likely that they do. That is, their effect on cholesterol metabolism could be related to the unsaturated nature of these fatty acids.

Spady and Dietschy (1988) found in their animal study that OA and LA diminished the suppressive effect of dietary cholesterol on receptor-dependent LDL transport. The International Collaborative Study Group (1986) suggested that OA may exert a hypocholesterolemic effect by increasing the LDL receptors activity. Shepherd et al. (1980) found that high LA consumption caused LDL fractional catabolic rates to increase with a decrease in LDL apo B synthesis. By contrast Cortese et al. (1983), after feeding hypercholesterolemic subjects a sunflower oil-rich diet found that the synthetic rates of both VLDL apo B and LDL apo B were reduced without any change in the LDL fractional catabolic rates. However,

this group of subjects could have been poor responders as identified by Grundy and Vega (1988). This trait also could explain the findings in the present study, i.e. some of the subjects were poor responders. Although hypercholesterolemia can be due to an abnormal gene for LDL receptors (Grundy, 1987), not all individuals with plasma cholesterol levels greater than 6.2 mmol/L have this genetic disorder, but they may have reduced LDL receptors activity (Grundy & Vega, 1985).

Grundy (1986, 1987) postulated that unsaturated fatty acids may lower plasma cholesterol simply by replacing SFA in the diet. However, Keys et al. (1957) and Hegsted et al. (1965) proposed that the hypocholesterolemic effect of PUFA is distinct from that due to just the replacement of dietary SFA. According to the equations of Keys et al. (1957) and Hegsted et al. (1965) SFA raised the TC about twice as much as LA lowered it. Nevertheless, the mechanisms via which unsaturated fatty acids lower cholesterol were not investigated by Grundy (1987), Hegsted et al. (1965) or Keys et al. (1957).

Although the mechanisms involved in plasma cholesterol metabolism have not been confirmed, a number of possibilities have been proposed. Spady and Dietschy (1988) stated that cholesterol metabolism is influenced by the absorption of cholesterol from the small intestine; rates of de novo cholesterol synthesis; the distribution of cholesterol between plasma and various extrahepatic pools; the cholesterol content

of VLDL and LDL; and the rates of synthesis or catabolism of plasma lipoproteins. Other authors (Kinsella, 1988; Nestel, 1987; Nestel et al., 1984) reported that unsaturated fatty acids decrease plasma cholesterol by improving receptor-mediated LDL uptake and enhancing cholesterol excretion via bile acids. Berlin et al. (1987) found that dietary LA was effective in increasing LDL fluidity. This diet-induced fluidity may affect the conformation of the apoprotein moiety on the surface of LDL and hence enhance the interaction between LDL and LDL receptors. This, in turn, improves the clearance of circulating LDL.

The results of the present study were not completely consistent with those found previously in our laboratory (Chan, 1990; McDonald et al., 1989) with normolipidemic subjects. The most consistent observation, however, was the fact that dietary fat source did not affect HDL-C level. Also, no changes were detected in the VLDL-C levels as observed previously by McDonald et al. (1989) following experimental diets which were high in OA or LA. However, in the study by Chan (1990), a drop in VLDL-C levels was observed.

The results of the present study and those of other investigators suggest that the design and methodology employed in any study can influence its outcome. The design and methodology of the study are affected by the nature of the diets, the number and type of subjects, and the duration of

the study. The design and methodology employed also affects the statistical methods that can be used to analyze the results. In other words, significant reductions in TC and LDL-C levels may have occurred for all experimental diets in the present study if the duration of the experimental periods had been longer. Likewise, the failure to find significant reductions in TC and LDL-C in response to the S/O and S/O/F diets at the end of Experimental Period I could be due to the conservative approach taken in the statistical analysis in the present study.

It is important to note that dietary fat source did not affect the level of HDL-C. This is fortunate since HDL-C level is inversely related to the risk of CHD (Castelli et al., 1986; Goldbourt & Yaari, 1990). Contrasting results were observed by Mattson and Grundy (1985), Shepherd et al, (1978, 1980) and Sirtori et al. (1986), who found that a decrease in HDL-C level was associated with diets rich in LA. The difference in results compared to the present study could be attributed to the lower LA and P/S ratio of the diets used in the present study.

There were no significant differences in the plasma TG levels among the diets used in the present study except for (i) the rise in the TG level in the SOY group when switched back to the mixed fat diet ($p < 0.05$) and (ii) the lower TG level for the S/O/F group than the other three groups, i.e. SOY ($p < 0.03$), CAN ($p < 0.04$) and S/O ($p < 0.02$) during

Experimental Period II. The latter was due to a drop in the TG level for the S/O/F group, while the TG levels for the other groups either remained constant or increased slightly. Some earlier studies (Mest et al., 1983; Renaud et al., 1986; Sanders & Roshanai, 1983) found that the effect of LNA on plasma TG was insignificant, while others (Baggio et al., 1988; Becker et al., 1983; Shepherd et al., 1978; Vega et al., 1982) found that the administration of vegetable oils had inconsistent influence on plasma TG levels. Thus the effect of OA, LA and LNA on plasma TG metabolism cannot be confirmed.

According to the results of the present study, it can be said that the CAN and SOY diets would be expected to have a positive impact on decreasing the risk of CHD. However, whether the S/O and S/O/F diets are as effective as the CAN and SOY diets cannot be concluded from the present study, since their cholesterol-lowering effect was not as dramatic as that observed following the CAN and SOY diets. On the other hand, since the CAN diet contained about twice as much OA and half as much LA as the SOY diet, while the LNA content was approximately the same in both diets, one can deduce that OA, LA and probably LNA have similar cholesterol-lowering effects. Although the mechanisms through which these fatty acids lower plasma cholesterol are not very clear, it is likely that they influence plasma cholesterol metabolism via similar mechanisms.

5.2 Plasma Phospholipid and Cholesteryl Ester Fatty Acid Patterns

Not surprisingly, dietary fat source had an influence on plasma PL and CE fatty acid patterns. This is indicated by the fact that plasma lipid OA, LA and LNA levels after the experimental diets reflected fairly closely the fatty acid composition of the diets. Interestingly, the level and magnitude of change in LNA was not great. Similar observations with respect to dietary LNA on the fatty acid patterns of plasma lipids have been documented (Adam et al., 1986; Chan, 1990; Ferreti et al., 1985; Renaud et al., 1986). On the other hand, increases in LNA consumption have been found to result in lower plasma LA levels (Budowski et al., 1984; Mest et al., 1983). Budowski et al. (1984) stated that the lower plasma LA levels were due to preferential incorporation of LNA into plasma lipids. However, preferential incorporation of LNA into plasma lipids was not found in the present study, nor was preferential incorporation of LNA found in the study by Chan (1990). No significant differences were observed in plasma LA levels following the S/O and CAN diets, although the latter had a higher LNA level. Similarly, the SOY and S/O/F diets, which had different LNA contents, did not differ significantly in their effects on plasma LA level except for two occasions, i.e. PC on Day 25 and CE on Day 43.

Although dietary fat source had an influence on plasma PL

and CE fatty acid patterns, the magnitude of change in plasma fatty acid levels was small compared to the variations in fatty acid composition in the diets. This small impact of the dietary fatty acids may be a result of several factors: (i) influence of lipoprotein metabolism on the fatty acid patterns of plasma lipids; (ii) limited incorporation of dietary fatty acids into plasma lipids; and (iii) activities of desaturases and elongases. Since fatty acids with chain lengths longer than 18 carbons were insignificant in the diets of the present study, the primary contribution of these longer chain PUFA in plasma lipids would likely be due to the desaturation and chain elongation of LA and LNA.

The results of the present study showed that following the CAN and S/O/F diets, consistently higher levels of LNA and EPA were noted in plasma lipids on both Days 25 and 43. In addition, the levels of EPA of plasma PL and CE were frequently more than double the levels found following the S/O and SOY diets. This suggests that the parent fatty acid of the n-3 family, i.e. LNA, was desaturated and elongated to EPA in the human body. Other studies (Adam et al., 1986; Budowski et al., 1984; Chan, 1990; Lasserre et al., 1985; Mest et al., 1983; Renaud et al., 1986; Sanders & Roshanai, 1983) also have proven that dietary LNA can be desaturated and elongated to EPA. However, Sanders and Roshanai (1983) and Sanders and Younger (1981) found that the conversion of LNA to EPA in vivo was less efficient in increasing EPA level in PL as direct EPA

consumption which resulted in more than a four-fold increase in plasma or platelet EPA levels.

Interestingly, the CAN and SOY diets, which had similar amounts of LNA, resulted in varied levels of EPA in plasma lipids. Hence, it becomes obvious that the in vivo synthesis of EPA is dependent on more than the availability of LNA in the diet. Judging by the high level of LA in the SOY diet, the high LA level could be the cause of lower EPA level after the SOY diet as compared to the CAN diet. It has also been reported that high LA consumption may hinder EPA production from LNA (Dyerberg, 1986; Leaf & Weber, 1988). This is likely due to the successful competition of LA over LNA for the same enzyme systems. Therefore, it is deduced that dietary LA/LNA ratio may play a crucial role with respect to the metabolism of n-3 PUFA and that the optimum LA/LNA ratio is yet to be determined such that the resulting effect on prostanoid metabolism would be positive. In short, the resulting effect should shift the prostanoid homeostasis towards a less thrombogenic state.

The CAN and S/O/F diets, which had similar LA/LNA ratios, i.e. 2.6/1 and 2.2/1, respectively, resulted in similar increases in plasma EPA levels. Similar results were observed last year in our laboratory by Chan (1990). The importance of dietary LA/LNA ratio has been studied and discussed by other investigators (Sanders & Younger, 1981; Marshall & Johnston, 1982). Sanders and Younger (1981) found that administering a

linseed oil supplement to omnivores which altered their dietary LA/LNA ratio from 6/1 to 1/1 resulted in a two-fold (1.3% to 2.7%) increase in plasma PC EPA level. The same investigators observed that when the dietary LA/LNA ratio of the vegetarians in the study was changed from 16/1 to 3/1 through the linseed oil supplement, a three-fold (0.3% to 1.0%) increase in plasma PC EPA level occurred. Thus, a low dietary LA/LNA ratio appeared to have a great influence on the n-3 PUFA metabolism in these subjects. Similar findings were observed by Marshall and Johnston (1982) when rats were fed diets which caused a lowering of the LA/LNA ratio to 0.3/1 and 1/1, i.e. the liver PE n-3 PUFA levels increased. By contrast, EPA levels in the same fraction dropped when the LA/LNA ratios were high, i.e. 7/1 and 32/1. The results of the present study were consistent with these findings; high LA/LNA ratios, i.e. 21/1 and 7/1, on the S/O and SOY diets, respectively, were associated with significantly lower levels of EPA in plasma PL and CE. Ziboh and Chapkin (1988) proposed that the increase of n-3 PUFA through a decreased LA/LNA ratio could be attributed to the suppressive effect of LNA over LA when competing for the Δ6-desaturase.

Both the SOY and S/O diets exerted the same influence on the n-3 PUFA metabolism in spite of the fact that the LA/LNA ratio of the SOY diet was much lower than that of the S/O diet. Perhaps having the LA/LNA ratio as high as 7/1, as in the present study, had a significant effect on the metabolism

of n-3 PUFA, but an increase to 21/1 had no additional effect. By contrast, the SOY and S/O/F diets were frequently associated with significantly lower levels of ETA in plasma lipids on Day 25 but not on Day 43. It is rather remarkable, since the SOY and S/O/F diets had different LA/LNA ratios but both had high levels of LNA. This means that the actual levels of dietary LNA seemed to affect the n-6 PUFA metabolism more drastically than the LA/LNA ratio in these diets. This is further confirmed by the fact that although the CAN diet had similar LA/LNA ratios as the S/O/F diet, and similar amounts of LNA as the SOY diet, the influence of the SOY and S/O/F diets on the n-6 PUFA metabolism was more profound than that exerted by the CAN diet. At this point in time, these variations in results cannot be clearly elucidated. A possible explanation was proposed by Ziboh and Chapkin (1988) regarding the lower ETA level after the S/O/F diet. It is likely that the high level of LNA in the S/O/F diet was responsible for the suppressive effect with respect to the n-6 PUFA metabolism. However, the same shift in n-6 PUFA metabolism was not observed after the CAN diet probably because of its lower LNA level compared to the S/O/F diet.

Lasserre et al. (1985) in a long-term study observed that when human subjects were fed a diet of 4.5% energy as LA and 1.5% as LNA, similar to the percentage of energy from LNA in the CAN and SOY diets in the present study (Appendix 4), the impact on plasma n-6 PUFA metabolism was similar to that of a

diet providing 6.5% energy as LA and an insignificant amount of LNA. Thus, it would seem that in order to lower n-6 PUFA in the plasma, the diet should supply more than 1.5% energy as LNA. A study by Sanders and Roshanai (1983) administering 20 ml/day of a linseed oil supplement (about 9 g/day LNA), which is similar to the amount of LNA in the CAN and SOY diets in the present study, found that the levels of platelet LNA and EPA increased, DTA level decreased and AA level remained constant. By contrast, Mest et al. (1983), who administered 30 ml/day of a linseed oil supplement (about 16 g/day LNA), which is similar to the amount of LNA in the S/O/F diet in the present study, found decreased plasma levels of LA and AA. Budowski et al. (1984) also reported significantly lower levels of plasma AA when the diet was supplemented with 60 ml/day linseed oil for six weeks. Chan (1990) also found that high levels of LNA were more likely to suppress plasma AA levels. The results of the present study and those of previous investigators suggest that a high level of dietary LNA, i.e. greater than 9 g/day, is likely to suppress the metabolism of plasma LA.

However, other findings also have been reported (Adam et al., 1986; Renaud et al., 1986). Adam et al. (1986) observed that a diet supplying up to 16% of calories as LNA and a LA/LNA ratio of 0.25/1 did not suppress plasma AA level. It could be that a two-week experimental trial was too short a duration for this level of dietary LNA to have any effect on

plasma AA level. By contrast, Renaud et al. (1986), found with a group of French farmers, when their normal diet was changed for one year to a diet with more LNA (1% of energy), which is about half the amount in the CAN and SOY diets (Appendix 4), that a significant decrease in plasma AA level occurred. Nevertheless, it is not entirely clear if the length of the experimental trial had a critical role in affecting the outcome of their studies.

The SOY and CAN diets in the present study had similar amounts of LNA. However, the SOY diet was associated with significantly lower levels of ETA as compared to the CAN diet. Thus, the ETA-lowering effect does not seem to be accounted for by the dietary LNA. Also, the SOY and S/O/F diets, which had higher levels of dietary LA than the CAN diet, were associated with lower levels of ETA than the CAN diet. It could be that the n-6 PUFA metabolism is affected by the level of dietary LA. Similar findings were reported by Chan (1990) with normolipidemic subjects. Lasserre et al. (1985) in a study with 24 female volunteers who were fed diets with different fat sources for five months found that when the diet supplied 14% of energy as LA and an insignificant level of LNA, which is similar to the percentage energy of LA in the SOY diet (Appendix 4), serum PL and CE levels of ETA and AA decreased significantly. However, when the diet supplied 6.5% of energy as LA, which is similar to the percentage energy of LA in the S/O and CAN diets (Appendix 4), serum levels of ETA

and AA increased. From these observations, it seems that a high level of dietary LA is likely to suppress the n-6 PUFA metabolism. The suppression of high levels of dietary LA on AA levels also was reported by Dupont (1987, 1990) and Kinsella (1986). This suppression could be due to the competition among the fatty acids for the 2-acyl position of the PL (Lasserre et al., 1985).

Contrasting results were found by Ferreti et al. (1985) when subjects were fed diets supplying 3% energy as LA, which is similar to the level of LA energy in the mixed fat diet (Appendix 4), or 8% energy as LA, which is similar to the level of LA energy in the S/O and CAN diets (Appendix 4). That is, the AA levels were not affected by LA in either diet. Similar findings were observed in the present study, such that the plasma AA levels had no significant differences between the mixed fat and the S/O and CAN diets. Similarly, Chan (1990) found there were no differences in plasma AA levels between the mixed fat and the S/O and CAN diets which contributed about the same percentage of calories from LA as described above. It could be that the plasma AA levels are influenced by more than just dietary LA. Renaud et al. (1986) in a long-term study found that when LA consumption was increased from 4.9% to 9.1% of calories, the levels of ETA and AA remained constant. According to the findings of the present study and those of other investigators (Chan, 1990; Ferreti et al., 1985; Lasserre et al., 1985; Renaud et al.,

1986), it would be quite appropriate to propose that dietary LA of less than 9% energy has little or no effect on n-6 PUFA metabolism, while a diet with about 14% energy as LA is likely to have a suppressive effect on the n-6 PUFA metabolism.

The effects of dietary LA and LNA on DHA are important because DHA may act as a potential storage form of EPA (Leaf & Weber, 1988). Dietary fat source had no significant effect on the levels of plasma DHA in the present study. Similar results were obtained by other investigators (Chan, 1990; Lasserre et al., 1985; Renaud et al., 1986; Sanders & Roshanai, 1983; Sanders & Younger, 1981). By contrast, Mest et al. (1983) found higher levels of plasma DHA after linseed oil supplementation. Sanders and Younger (1981) proposed that the failure of linseed oil supplementation to increase the level of DHA could be due to the low activity of the Δ4-desaturase in humans, although it does not explain the findings of Mest et al. (1983).

Budowski et al. (1984) found that a minor change in plasma EPA after linseed oil supplementation resulted in a striking decrease in platelet sensitivity to collagen. These results coincide with the findings of Renaud et al. (1986) where diet modification on French farmers, by decreasing the amount of SFA and increasing the amounts of LA and LNA, resulted in a significant drop in plasma AA level (7.2% to 6.4% of total fatty acids) and a significant rise in plasma EPA level (0.6% to 0.7% of total fatty acids). Although there

were only minor changes in the plasma lipids, there was an obvious positive impact on platelet aggregation and bleeding time. Thus, the small differences in the plasma fatty acid levels found in the present study could have had a significant influence on platelet function and bleeding time in hyperlipidemic subjects.

The results of the present study indicated that dietary OA, LA and probably LNA have similar hypocholesterolemic effects in hyperlipidemic subjects. However, it is still unclear if these fatty acids affect lipoprotein metabolism via similar mechanisms. Dietary fat source had an influence on plasma fatty acid patterns although the magnitude of the effect was not great. LNA can be desaturated and elongated to EPA in hyperlipidemic individuals. The dietary LA/LNA ratio appeared to have a great influence on the n-3 PUFA metabolism, while the absolute levels of dietary LA and LNA seemed to exert a greater impact on the n-6 PUFA metabolism in the hyperlipidemic individuals.

6. SUMMARY AND CONCLUSIONS

Past research studies have shown that dietary fatty acids are capable of influencing the risk of CHD. CHD is the leading cause of morbidity and mortality in western industrialized nations such as Canada. The two major degenerative events in CHD are atherosclerosis and thrombosis. It has been very well established that dietary SFA are hypercholesterolemic, while OA and LA are hypocholesterolemic. The hypocholesterolemic effect of LNA needs further clarification. Since most of the previous studies on lipid metabolism were investigated using normolipidemic subjects, it would be interesting to see if the effects of dietary fatty acids on lipid metabolism would have the same impact in hyperlipidemic individuals.

The purpose of the present study was to investigate the effects of diets varying in SFA, OA, LA and LNA on the plasma lipid and lipoprotein metabolism and the plasma lipid fatty acid patterns in a group of individuals with mildly elevated cholesterol levels (≥5.8 mmol/L).

The study was comprised of two 48-day replicates. Each replicate was divided into four periods: a 6-day pre-experimental; two 18-day experimental; and a 6-day post-experimental period. During the pre- and post-experimental periods, all subjects were given a mixed fat diet. During the experimental periods, two subjects were randomly assigned to one of the following diets: S/O, CAN, SOY and S/O/F diets.

On Day 25, each pair of subjects was switched to a different diet according to this order: S/O to SOY; SOY to CAN; CAN to S/O/F; S/O/F to S/O. All the diets were identical except for the sources of added fat. The diets, providing approximately 3200 kcal/day, were constituted of macronutrients in the proportions of 51% carbohydrate, 13% protein and 36% fat. The sources of added fat accounted for approximately 81% of the total fat in the diet. The experimental diets had about half the level of SFA as in the mixed fat diet, while the percentages of OA, LA and LNA of the S/O, CAN, SOY and S/O/F diets were 58, 19 and 1; 54, 19 and 8; 25, 45 and 7; and 31, 33 and 15, respectively. A 12-hour overnight venous blood sample was drawn at the beginning and the end of each experimental period. Plasma TC, LDL-C, HDL-C, VLDL-C and TG levels were analyzed on fresh plasma samples while the fatty acid patterns of plasma PC, PE, PPE and CE were analyzed on properly stored plasma samples.

A significant reduction ($p < 0.05$) in mean plasma TC and LDL-C levels was observed in the CAN and SOY groups at the end of Experimental Period I. A similar pattern of reduction was observed for subjects fed the S/O and S/O/F diets, but the reductions in mean plasma TC and LDL-C levels were not significantly different for these two groups. The decrease in TC level was attributable mainly to the drop in LDL-C level. Dietary fat source had no effect on HDL-C and VLDL-C levels, while the TG level was significantly lower after the S/O/F

diet than with the other three diets.

The experimental diets varied in their effects on plasma PL and CE fatty acid patterns. Diets which contained relatively high amounts of OA (S/O and CAN diets), LA (SOY and S/O/F diets) and LNA (CAN, SOY and S/O/F diets) were usually associated with significantly higher levels of plasma OA, LA and LNA, respectively. The SOY and S/O/F diets were associated with lower levels of ETA, whereas the CAN and S/O/F diets were associated with higher levels of EPA in the plasma lipids.

Results of the present study indicated that dietary OA, LA, and probably LNA had similar cholesterol-lowering effects in hyperlipidemic subjects; dietary fat source had an appreciable effect on plasma lipid fatty acid patterns; and dietary LNA is desaturated and elongated to EPA in the human body. There also was an indication that dietary LA/LNA ratio had an influence on n-3 PUFA metabolism, while the absolute amounts of dietary LA and LNA had a greater impact on n-6 PUFA metabolism in the hyperlipidemic individuals.

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APPENDICES

Appendix 1. Consent Form

HUMAN NUTRITION RESEARCH PROJECT

DEPARTMENT OF FOODS AND NUTRITION

UNIVERSITY OF MANITOBA

CONSENT FORM

I understand that I have been selected for the metabolic study because of an elevation in my serum cholesterol.

As a volunteer in the research project designed to study the effect of polyunsaturated fatty acids on serum lipids, platelet function and metabolism, I am aware of the nature of the problem being investigated. I acknowledge that I have been briefed in the project protocol and I am aware of the requirements to be fulfilled by the subjects.

I am aware that blood samples will be collected at intervals throughout the study. I understand that in addition to venous blood samples, a small cut, less than 1/4 inch will be made that may leave a small scar and that the tests will require the bleeding to continue for 5 to 15 minutes. I understand that I will have the cut made three times during the research study.

I understand that a physical examination is required and that the costs of the examination are assumed by the investigator.

Dated_____day of_____19____.

Signature_____.

Witness_____.

Appendix 2. Fatty Acid Composition of the Oils Used During the Experimental Periods[1]

Fatty Acid[2]	Oils				
	Sunflower	Olive	Soybean	Canola	Flax
	------	% of total fatty acids			------
14:0	0.1	-	0.1	0.1	-
16:0	6.0	9.0	10.5	4.3	5.3
16:1	0.1	0.4	0.1	0.2	-
18:0	4.6	2.6	3.5	2.0	2.9
18:1	14.5	75.6	22.5	62.0	20.4
18:2	73.1	10.0	53.8	20.1	13.8
18:3	0.4	0.8	8.4	8.0	57.2
20:0	0.3	0.6	0.4	0.8	0.1
20:1	0.2	0.6	0.3	1.7	0.2
22:0	0.8	0.4	0.4	0.4	0.1
22:1	-	-	-	0.3	-

[1] Means of two samples
[2] Carbon chain length : number of double bonds

Appendix 3. Fatty Acid Composition of the Diets[1]

Fatty Acid[2]	Diets				
	Mixed Fat	S/O	CAN	SOY	S/O/F
	------	% of total fatty acids			------
14:0	4.4	1.3	1.3	1.4	1.5
16:0	24.0	12.6	8.9	14.2	11.0
16:1	1.7	0.8	0.5	0.4	0.5
18:0	12.1	4.6	3.5	4.9	5.3
18:1	39.2	58.3	54.0	24.9	30.9
18:2	12.3	18.8	19.4	44.9	33.3
18:3	0.9	0.9	7.5	6.5	15.0
20:0	0.3	0.4	0.6	0.3	0.3
20:1	0.5	0.4	1.5	0.3	0.3
22:0	0.1	0.2	0.3	0.3	0.4
22:1	0.0	0.0	0.4	0.0	0.1

[1] Means of four duplicate diet samples (two of each menu)
[2] Carbon chain length : number of double bonds

Appendix 4. Contribution of Saturated, Oleic, Linoleic and Linolenic Acids to Total Energy Intake in Different Diets[1]

Fatty Acid	Diets				
	Mixed Fat	S/O	CAN	SOY	S/O/F
		------	% calories	------	
SFA	14.6	6.8	5.2	7.5	6.6
OA	14.0	20.8	19.2	8.9	11.0
LA	4.4	6.7	6.9	16.0	11.9
LNA	0.3	0.3	2.7	2.3	5.3

[1] Means of four duplicate samples (two of each menu)

Appendix 5. Recipes

Granola — Yield: 58 x 40 g servings

1 kg	rolled oats
125 g	bran
700 g	oil/fat mix[1]
500 g	brown sugar

Preheat oven to 350° F.
Combine all ingredients.
Toast in oven for approximately 15 minutes or until golden brown.
Weigh into 40 g individual portions and package.

Oatmeal Cookies — Yield: 30 cookies

215 g	sifted pastry flour
3 g	salt
190 g	rolled oats
180 g	oil/fat mix[1]
150 g	brown sugar
4 ml	vanilla
4.5 g	baking soda
50 ml	boiling water

Preheat oven to 350° F.
Place sifted flour and salt in a large bowl and combine well.
Mix in rolled oats.
Combine oil, brown sugar and vanilla in a small bowl.
Dissolve baking soda in boiling water and stir into oil mixture.
Combine wet and dry ingredients, mixing thoroughly.
Weigh out individual cookies - 25 g each.
Place on ungreased cookie sheet and flatten with a fork into a round cookie.
Bake for 15 minutes or until golden brown.

Baked Rice — Yield: 1 serving

30 g	rice
9 or 10 g	oil/fat mix[1]
70 g	boiling water
1 ml	salt

Place rice, boiling water and salt into individual casseroles.
Cover and bake at 350° F for 30 minutes.
Add oil after cooking to hot rice.

. . . cont'd

Appendix 5 (cont'd).

Jellied Fruit — Yield: 1 serving

100 g	jello (prepared)
100 g	canned fruit

Prepare jello according to package directions.
Place fruit and jello into individual serving dishes.
Refrigerate.

Mashed Potatoes — Yield: 1 serving

30 g	potato flakes
50 ml	skim milk
22, 24 or 26 g	oil/fat mix[1]
1 ml	salt
100 ml	boiling water

Put potato flakes, milk, oil and salt into individual casseroles.
Stir in boiling water until the mixture reaches a creamy and fluffy consistency.

Carrots — Yield: 1 serving

100 g	frozen carrots
5 or 6 g	oil/fat mix[1]

Place carrots in casserole.
Microwave on high for 3 minutes.
Add in oil.
Let stand 5 minutes. Reheat.

Chili — Yield: 1 serving

60 g	cooked ground beef
18 or 21 g	oil/fat mix[1]
120 g	canned tomatoes, drained
65 g	kidney beans, drained
3 g	dehydrated onions
1.5 ml	Worcestershire sauce
2 ml	chili powder

Put ingredients into individual casseroles. Mix well.
Bake at 350° F for 30 minutes.
OR microwave on medium-high for 4-5 minutes.

. . . cont'd

Appendix 5 (cont'd).

Chicken Casserole — Yield: 1 serving

60 g	cooked chicken breast, in small pieces
13, 14 or 16 g	oil/fat mix[1]
30 g	celery
40 g	canned mushrooms, drained
40 g	frozen peas
20 g	chicken broth

Put ingredients in individual casseroles.
Cover and bake at 350° F for 20 minutes.
OR microwave on medium-high for 3 minutes.
Add oil.
Reheat.

Noodles — Yield: 1 serving

30 g	noodles
175 ml	boiling water
5 ml	oil
5 g	oil/fat mix[1]

Place noodles, water and 5 ml of oil into individual bowls.
Stir. Microwave on high for 3 minutes.
Stir. Let stand for 2 minutes.
Drain. Transfer to individual casseroles.
Press fork on noodles. (Fork marks are visible on noodles when done.)
Stir in remaining oil.
Can be reheated.

---

[1] See Table 5 for the amounts and types of oil or fat used in different diets.

Appendix 6. GC Conditions for Diet and Plasma Lipid Analysis

Gas Chromatograph: Hewlett Packard 5890
Integrator: Hewlett Packard 3392A
Column: DB-225 capillary column, 30 m x 0.25 mm, film thickness 0.25 microns, J & W Scientific (from Chromatographic Specialties)

GC Conditions:
- injector and detector temperatures 250°C
- oven temperature 205°C, isothermal
- column head pressure 18 psi
- He flow 50 ml/min (set to 17 ml/min overnight)
- gas gauge settings: Air 50 psi
  Hydrogen 50 psi
  Nitrogen 60 psi
  Helium 50 psi
- other GC settings: Range 2
  Zero OFF
  Attenuation 0
  Purge B ON
  Oven max 230°C

Appendix 7. Integrator Programs for Diet and Plasma Lipid Analysis

(A) Integrator Program for Diet Analysis

```
RUN PRMTRS
ZERO    =  10
ATT 2↑ =  1
CHT SP =  0.5
PK WD   =  0.04
THRSH   =  -1
AR REJ =  2000

RPRT OPTNS
  2. RF UNC PKS=       0.0000E+00
  3. MUL FACTOR=       1.0000E+00
  4. PK HEIGHT MODE       NO
  5. EXTEND RT            NO
  6. RPRT UNC PKS         NO

TIME TBL
   0.00 INTG # =  9            (disable integration)
   0.10 INTG # =  8            (delete start/stop tick marks)
   3.00 INTG # =  -9           (start integration)
  29.00 STOP
```

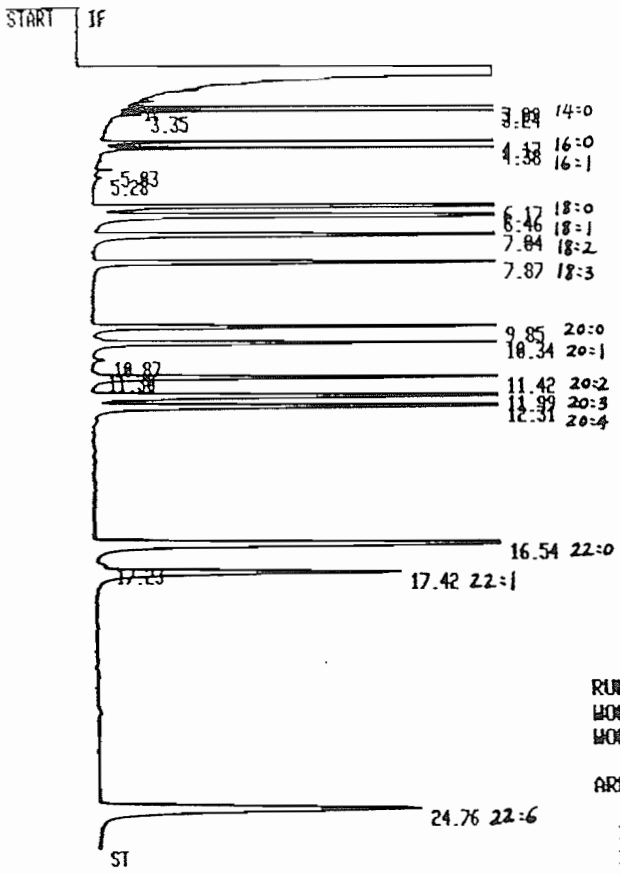
(B) Integrator Program for Plasma Lipid Analysis

```
RUN PRMTRS
ZERO    =  10
ATT 2↑ =  0
CHT SP =  0.5
PK WD   =  0.04
THRSH   =  -1
AR REJ =  0

RPRT OPTNS
  2. RF UNC PKS=       0.0000E+00
  3. MUL FACTOR=       1.0000E+00
  4. PK HEIGHT MODE       NO
  5. EXTEND RT            NO
  6. RPRT UNC PKS         NO

TIME TBL
   0.00 INTG # =  9
   0.10 INTG # =  8
   3.00 INTG # =  -9
  26.00 STOP
```

Appendix 8. Chromatograms for the Fatty Acid Standards.
A. Chromatogram for the Standard Mix.



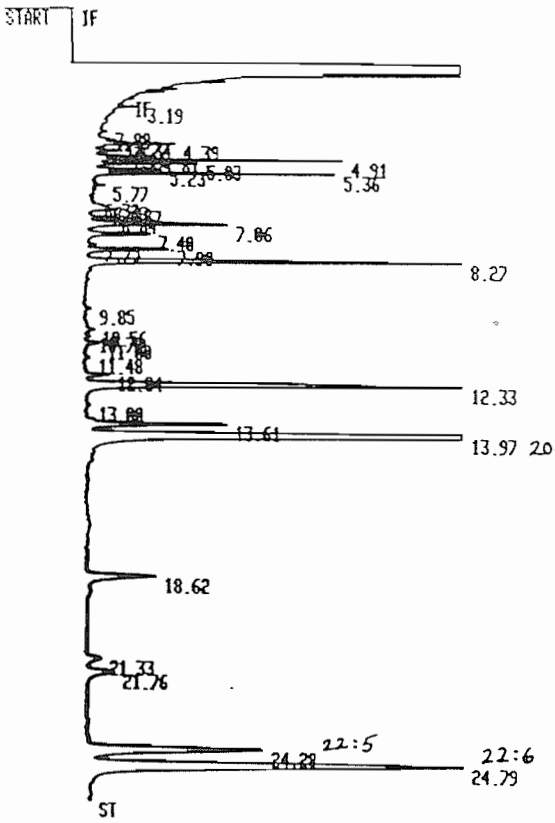
RUN # 101 NOV/24/90 14:57:36
WORKFILE ID: C
WORKFILE NAME:

AREA%

RT	AREA	TYPE	AR/HT	AREA%
3.89	70284	PB	0.027	3.122
3.24	22388	BB	0.028	0.991
3.35	532	BP	0.023	0.024
4.17	242730	PB	0.033	10.782
4.38	47434	PB	0.035	2.107
5.83	669	PV	0.043	0.030
5.28	451	BP	0.078	0.020
6.17	373160	PB	0.046	16.575
6.46	617680	PB	0.047	27.437
7.04	241170	PB	0.053	10.712
7.87	91000	PB	0.060	4.043
9.85	49730	PB	0.077	2.209
10.34	50773	BB	0.083	2.255
10.87	1002	PV	0.095	0.040
11.30	206	PV	0.040	0.009
11.42	47771	VB	0.091	2.122
11.99	89302	BV	0.091	3.967
12.31	85589	VB	0.090	3.002
16.54	102030	BB	0.131	4.532
17.23	914	PV	0.101	0.041
17.42	47903	VB	0.150	2.120
24.76	68575	PB	0.203	3.046

TOTAL AREA= 2251300
MUL FACTOR= 1.0000E+000

Appendix 8 (cont'd).
B. Chromatogram for the Eicosapentaenoic Acid (20:5) Standard.



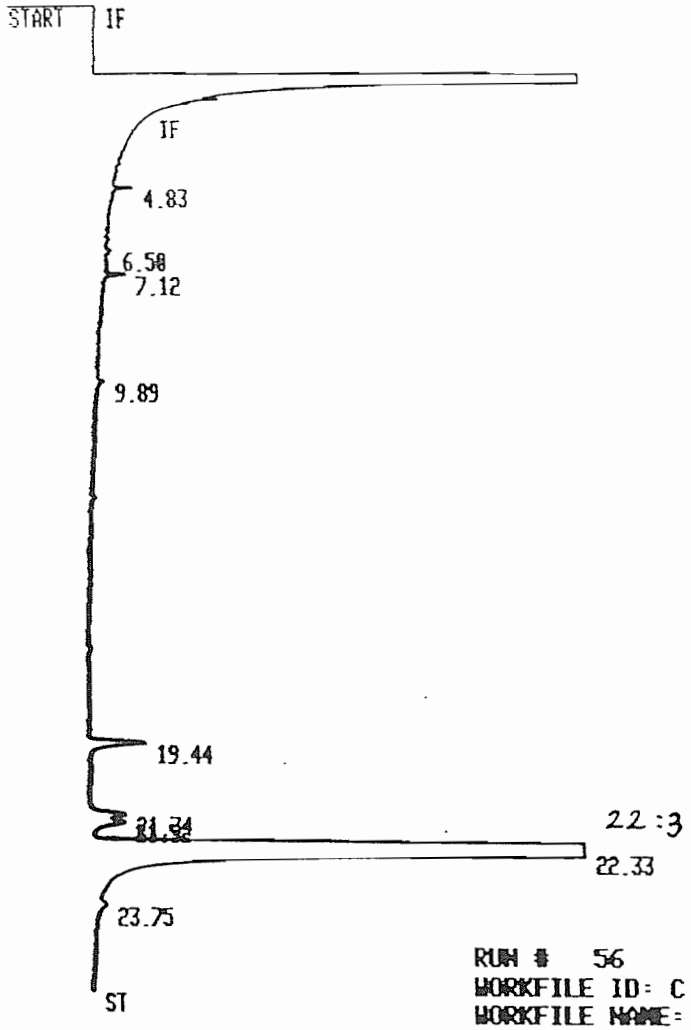
RUN # 55 NOV/16/98 12:32:37
WORKFILE ID: C
WORKFILE NAME:

AREA%

RT	AREA	TYPE	AR/HT	AREA%
3.19	996	PP	0.040	0.130
3.99	317	PP	0.049	0.041
4.27	1301	PV	0.067	0.169
4.34	1372	VV	0.042	0.179
4.39	3772	VV	0.043	0.491
4.61	1806	PV	0.065	0.235
4.81	2843	VV	0.043	0.370
4.91	12576	VV	0.046	1.636
5.03	5251	VV	0.046	0.683
5.23	4677	VV	0.065	0.608
5.36	13080	VB	0.049	1.701
5.77	626	VP	0.054	0.081
6.32	227	PV	0.050	0.030
6.43	892	VV	0.060	0.116
6.48	1012	VV	0.051	0.132
6.57	2684	VP	0.090	0.349
6.84	1921	PV	0.074	0.250
7.06	13974	VV	0.090	1.818
7.40	5453	VV	0.079	0.709
7.75	680	PV	0.069	0.089
7.90	7121	VP	0.078	0.926
8.27	56395	PB	0.069	7.335
9.85	609	PV	0.091	0.079
10.56	1050	VV	0.107	0.137
10.77	373	VV	0.074	0.049
11.00	1735	PV	0.105	0.226
11.48	626	PP	0.111	0.081
12.04	2448	VV	0.091	0.318
12.33	50765	VB	0.099	6.603
13.00	459	BP	0.102	0.060
13.61	16660	PP	0.100	2.167
13.97	397260	PB	0.105	51.671
18.62	12030	VV	0.156	1.566
21.33	3116	PV	0.194	0.405
21.76	5922	VV	0.194	0.770
24.28	36765	PV	0.109	4.702
24.79	100030	VB	0.197	13.010

TOTAL AREA= 768830
MUL FACTOR= 1.0000E+00

Appendix 8 (cont'd).
C. Chromatogram for the Docosatrienoic Acid (22:3) Standard.



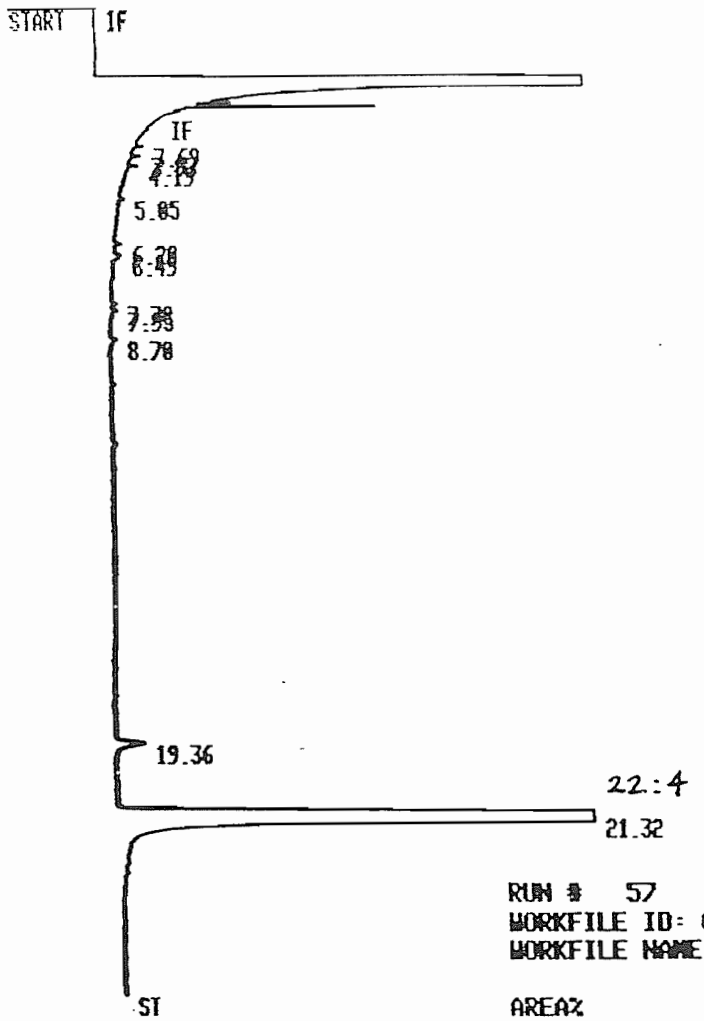
RUN # 56 NOV/16/98 13:10:14
WORKFILE ID: C
WORKFILE NAME:

AREA%

RT	AREA	TYPE	AR/HT	AREA%
4.83	1029	BV	0.059	0.057
6.58	257	PV	0.054	0.014
7.12	1193	VV	0.067	0.066
9.89	516	VV	0.087	0.028
19.44	8411	PV	0.162	0.462
21.34	5340	PV	0.173	0.293
21.52	5443	VV	0.185	0.299
22.33	1796800	PB	0.198	98.716
23.75	1184	BV	0.178	0.065

TOTAL AREA= 1820200
MUL FACTOR= 1.0000E+00

Appendix 8 (cont'd).
D. Chromatogram for the Docosatetraenoic Acid (22:4) Standard.



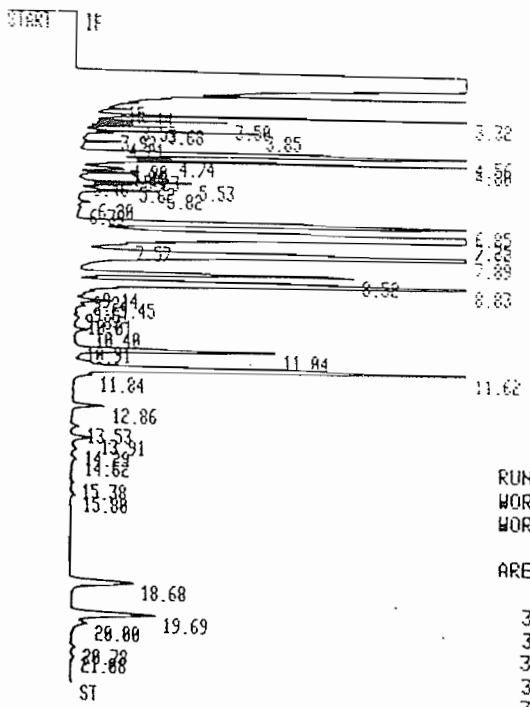
RUN # 57 NOV/16/90 13:47:28
WORKFILE ID: C
WORKFILE NAME:

AREA%

RT	AREA	TYPE	AR/HT	AREA%
3.69	443	PV	0.054	0.043
3.93	326	PB	0.038	0.031
4.19	300	BP	0.036	0.029
5.85	224	BB	0.052	0.022
6.20	440	PV	0.072	0.042
6.49	483	VV	0.076	0.046
7.78	368	VP	0.089	0.035
7.95	387	PV	0.090	0.037
8.70	554	PV	0.094	0.053
19.36	4154	PP	0.167	0.398
21.32	1035600	PB	0.165	99.264

TOTAL AREA= 1043300
MUL FACTOR= 1.0000E+00

Appendix 9. A Sample Fatty Acid Chromatogram for the Diet Analysis (Canola Diet, Menu I).



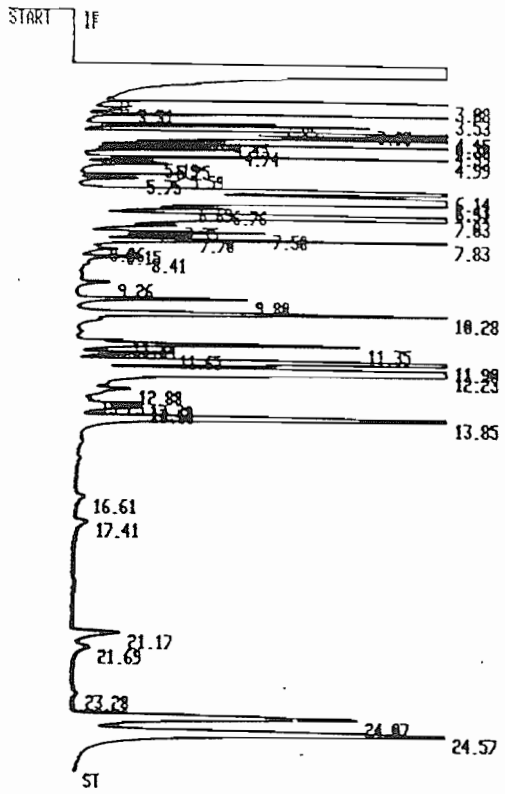
RUN # 288 MAY/08/90 12:57:28
WORKFILE ID: C
WORKFILE NAME:

AREA%

RT	AREA	TYPE	AR/HT	AREA%	
3.32	100850	BB	0.032	1.391	14:0
3.50	9424	BV	0.032	0.130	
3.59	3005	VP	0.034	0.042	
3.68	5307	PB	0.034	0.073	
3.85	13547	BV	0.035	0.187	
3.92	2486	VV	0.043	0.034	
4.21	3333	PV	0.039	0.046	
4.56	673100	PB	0.041	[9.284	
4.74	3722	BV	0.033	0.051]	16:0
4.80	39218	VV	0.040	0.541	16:1
5.06	3124	PV	0.038	0.043	
5.10	3986 D	VP	0.044	0.055	
5.23	6017	PP	0.050	0.083	
5.53	11944	VV	0.048	0.165	
5.62	6031	VV	0.054	0.083	
5.82	10459	VB	0.060	0.144	
6.85	253410	PB	0.064	3.495	18:0
7.25	3652700	PV	0.075	[50.383	
7.32	248830	VB	0.048	3.432]	18:1
7.89	1393600	PB	0.066	19.223	18:2
8.52	46359	BV	0.076	0.640	
8.83	516710	VV	0.076	7.127	18:3
9.14	2966	VV	0.081	0.041	
9.45	6509	VV	0.084	0.090	
9.61	2269	VP	0.114	0.031	
10.40	3185	PV	0.110	0.044	
11.04	43312	VV	0.096	0.597	20:0
11.62	105790	VV	0.105	1.459	20:1
11.84	4881	VV	0.119	0.067	
12.86	7292	VV	0.103	0.101	
13.91	6021	PV	0.125	0.083	
18.68	22745	PV	0.163	0.314	22:0
19.69	31013	VV	0.164	0.428	22:1
20.00	6682	VV	0.184	0.092	

TOTAL AREA= 7249900
MUL FACTOR= 1.0000E+00

Appendix 10. A Sample Fatty Acid Chromatogram for Plasma Phosphatidylcholine (Subject 3, Replicate I, Day 43).



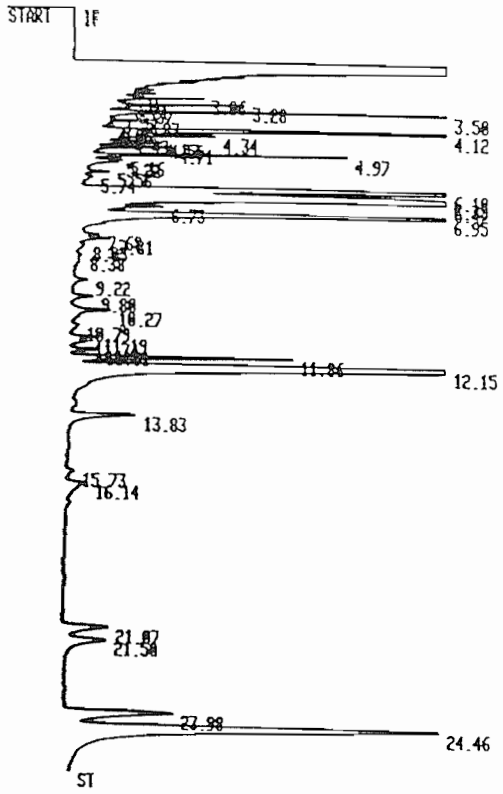
RUN # 428 JAN/18/91 18:14:08
WORKFILE ID: C
WORKFILE NAME:

AREA%

RT	AREA	TYPE	AR/HT	AREA%	
3.08	18207	BB	0.038	0.215	
3.31	1690	VV	0.043	0.020	
3.37	2131	VP	0.053	0.025	
3.53	45229	PB	0.032	0.535	15:0
3.85	7343	PV	0.033	0.087	
3.93	11352	VB	0.035	0.134	
4.15	2046900	PB	0.035	24.214	16:0
4.30	13119	BV	0.035	[0.155	
4.36	13641	VP	0.035	0.161]	16:1
4.45	5769	PP	0.051	0.068	
4.59	17949	PV	0.041	0.212	
4.74	7784	VP	0.046	0.092	
4.99	51281	PB	0.044	0.607	
5.19	3856	BV	0.050	0.046	
5.25	5646	VP	0.060	0.067	
5.59	5897	PV	0.050	0.070	
5.75	4184	VP	0.062	0.050	
6.14	1096300	PB	0.049	12.969	18:0
6.43	[1159600	BV	0.052	[13.718	
6.51	277610]	VB	0.051	3.284]	18:1
6.69	1093	BV	0.038	0.013	
6.76	4740	VP	0.051	0.056	
7.03	2214300	PB	0.050	26.194	18:2
7.35	2478	BP	0.043	0.029	
7.58	12786	PV	0.064	0.151	
7.78	7992	VV	0.060	0.095	
7.83	47352	VV	0.076	0.560	18:3
8.06	1113	VV	0.060	0.013	
8.15	3344	VP	0.090	0.040	
8.41	6220	PV	0.090	0.074	
9.26	3780	VV	0.103	0.045	
9.80	17340	VB	0.080	0.205	20:0
10.28	55854	BV	0.094	0.661	20:1
11.24	3710	PV	0.071	0.044	
11.35	31437	VV	0.096	0.372	20:2
11.65	9316	VP	0.090	0.110	
11.90	159300	PP	0.089	1.884	20:3
12.23	741270	PB	0.092	8.769	20:4
12.80	5695	PP	0.142	0.067	
13.25	434	PV	0.076	0.005	
13.38	6290	VV	0.100	0.074	
13.53	7583	VP	0.117	0.090	
13.85	100300	PB	0.122	1.187	20:5
16.61	1902	VV	0.196	0.023	
17.41	2823	PV	0.210	0.033	
21.17	11401	PV	0.207	0.135	22:4
21.69	5303	VV	0.265	0.063	—22:3
23.28	890	VV	0.181	0.011	
24.07	68225	BV	0.207	0.807	22:5
24.57	123730	VV	0.252	1.464	22:6

TOTAL AREA= 8453400
MUL FACTOR= 1.0000E+00

Appendix 11. A Sample Fatty Acid Chromatogram for Plasma Phosphatidylethanolamine (Subject 4, Replicate I, Day 7).



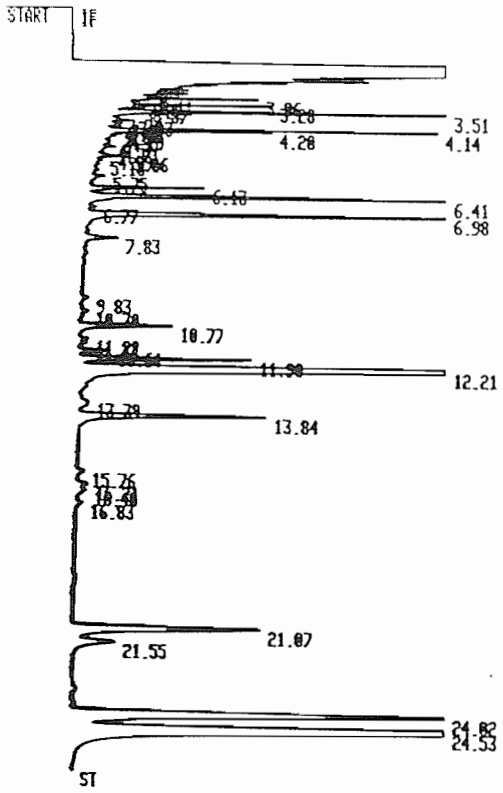
RUN # 10 FEB/08/91 19:01:08
WORKFILE ID: B
WORKFILE NAME:

AREA%

RT	AREA	TYPE	AR/HT	AREA%	
3.06	3264	BP	0.037	0.137	
3.21	415	PV	0.039	0.017	
3.28	7002	VV	0.046	0.293	
3.37	957	D VP	0.049	0.040	
3.50	173640	PB	0.037	7.264	15:0
3.73	280	BV	0.047	0.012	
3.83	1546	VV	0.045	0.065	
3.99	550	VP	0.049	0.023	
4.06	45	PP	0.022	0.002	
4.12	374160	PB	0.041	15.653	16:0
4.34	3673	BP	0.041	0.154	16:1
4.43	289	PP	0.033	0.012	
4.53	1962	PV	0.034	0.082	
4.56	3261	D VP	0.051	0.136	
4.71	4563	PP	0.054	0.191	
4.97	15970	PV	0.054	0.668	
5.16	1986	VV	0.057	0.083	
5.22	2422	VP	0.078	0.101	
5.56	1607	PP	0.064	0.067	
5.74	732	PV	0.085	0.031	
6.10	688540	PB	0.053	25.458	18:0
6.38	[234770]	BV	0.064	[9.822]	18:1
6.47	[25784]	D VP	0.068	[1.079]	
6.73	3139	PP	0.063	0.131	
6.95	286888	PB	0.062	11.968	18:2
7.68	1518	BV	0.083	0.064	
7.81	2965	VV	0.091	0.124	18:3
8.03	507	VV	0.085	0.021	
8.38	689	PV	0.132	0.029	
9.22	2155	VP	0.136	0.090	
9.88	2820	VV	0.119	0.118	20:0
10.27	7724	VV	0.173	0.323	20:1
10.79	884	VV	0.090	0.037	
11.19	2769	PV	0.098	0.116	
11.34	1907	VP	0.105	0.080	20:2
11.61	2747	PP	0.094	0.115	
11.86	23237	PP	0.092	0.972	20:3
12.15	388640	PB	0.104	16.259	20:4
13.83	11491	PV	0.156	0.481	20:5
15.73	1217	PV	0.157	0.051	
16.14	5327	VV	0.232	0.223	
21.07	10647	VV	0.285	0.445	22:4
21.58	12404	VV	0.257	0.519	22:3
23.98	27839	VV	0.219	1.165	22:5
24.46	126230	VV	0.289	5.281	22:6

TOTAL AREA= 2390408
MUL FACTOR= 1.0000E+00

Appendix 12. A Sample Fatty Acid Chromatogram for Plasma Alkenylacyl Ethanolamine Phosphoglyceride (Subject 12, Replicate II, Day 43).



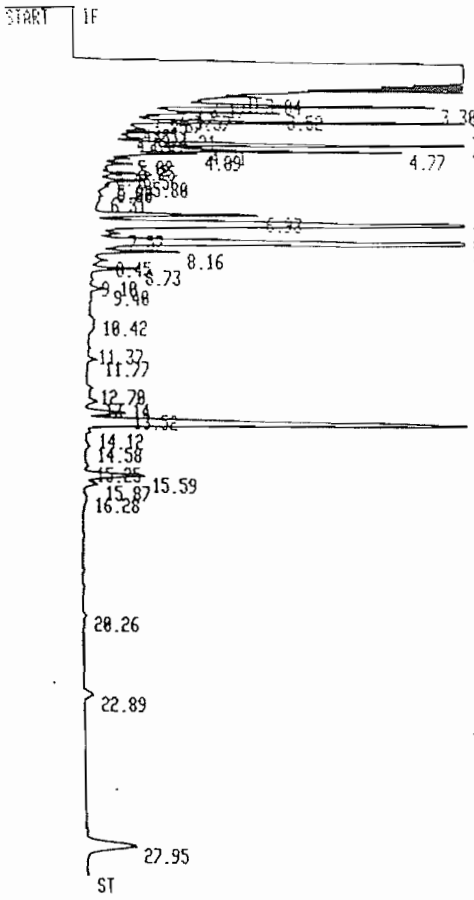
RUN # 245 DEC/24/98 16:28:21
WORKFILE ID: C
WORKFILE NAME:

AREA%

RT	AREA	TYPE	AR/HT	AREA%	
3.06	4836	BV	0.030	0.296	
3.11	483	D VP	0.025	0.030	
3.22	480	VV	0.036	0.035	
3.28	7534	VV	0.044	0.553	
3.37	1071	D VP	0.040	0.079	
3.51	227150	PB	0.033	16.665	15:0
3.74	223	BV	0.035	0.016	
3.83	1059	VV	0.052	0.078	
3.91	588	VV	0.047	0.043	
4.01	653	VP	0.053	0.048	
4.07	231	PV	0.032	0.017	
4.14	[14172	VV	0.037	[1.040	16:0
4.20	8363]	VV	0.044	0.614]	
4.29	701	VV	0.042	[0.051	16:1
4.35	661	VV	0.059	0.049]	
4.57	785	VV	0.038	0.058	
4.73	1058	VV	0.071	0.078	
4.99	482	PV	0.042	0.035	
5.06	1567	VV	0.050	0.115	
5.18	435	VV	0.078	0.032	
5.75	859	PV	0.063	0.063	
6.13	8082	VV	0.061	0.593	18:0
6.41	24874	VV	0.056	1.825	18:1
6.77	583	VP	0.063	0.037	
6.98	128190	PB	0.059	9.405	18:2
7.83	3735	VV	0.102	0.274	18:3
9.83	861	PV	0.106	0.063	20:0
10.30	853	VV	0.099	0.063	20:1
10.77	10453	PB	0.097	0.767	
11.22	790	BV	0.090	0.058	
11.37	713	VP	0.092	0.052	20:2
11.64	3000	PP	0.090	0.220	
11.90	17873	PP	0.091	1.311	20:3
12.21	523450	PB	0.094	38.404	20:4
13.39	460	PV	0.090	0.034	
13.84	26582	BB	0.122	1.950	20:5
15.76	1405	PV	0.176	0.103	
16.21	1720	VV	0.141	0.127	
16.40	1842	VV	0.179	0.135	
16.83	884	VP	0.135	0.065	
21.07	39250	BV	0.180	2.000	22:4
21.55	9933	VV	0.200	0.729	22:3
24.02	93220	PV	0.191	6.040	22:5
24.53	191820	VB	0.200	14.073	22:6

TOTAL AREA= 1363000
MUL FACTOR= 1.0000E+00

Appendix 13. A Sample Fatty Acid Chromatogram for Plasma Cholesteryl Esters (Subject 13, Replicate II, Day 7).



RUN # 330 AUG/08/90 11:31:19
WORKFILE ID: C
WORKFILE NAME:

AREA%

RT	AREA	TYPE	AR/HT	AREA%	
3.04	3502	BV	0.040	0.140	
3.30	24815	VB	0.043	0.993	
3.52	10567	VV	0.037	0.423	
3.57	3018 D	VV	0.037	0.121	
3.83	111060	VB	0.041	4.442	15:0
4.11	2733	VV	0.067	0.109	
4.21	4580	VP	0.043	0.183	
4.41	2159	VP	0.044	0.086	
4.56	270970	PB	0.048	10.839	16:0
4.71	3966	BV	0.037	[0.159	
4.77	26100	VV	0.046	1.044]	16:1
4.89	11765	VP	0.079	0.471	
5.08	2405	PP	0.057	0.096	
5.25	3713	PV	0.064	0.149	
5.56	5271	PV	0.072	0.211	
5.80	7064	VV	0.073	0.283	
6.00	2205	VP	0.111	0.088	
6.93	28266	PP	0.080	1.131	18:0
7.21	430420	PB	0.083	17.217	18:1
7.57	2647	BP	0.075	0.106	
7.84	1268300	PB	0.078	50.730	18:2
8.16	12283	BP	0.071	0.491	
8.45	2918	PP	0.102	0.117	
8.73	8676	PB	0.087	0.347	18:3
9.48	3316	PV	0.095	0.133	
11.77	2843	VP	0.134	0.114	
13.14	2659	VP	0.135	0.106	
13.52	11804	PV	0.134	0.472	20:3
13.81.	172730	VV	0.132	6.909	20:4
15.59	19508	VV	0.145	0.780	20:5
15.87	4675	VP	0.160	0.187	
22.89	4157	PP	0.217	0.166	
27.95	28951	VV	0.261	1.158	22:6

TOTAL AREA= 2500000
MUL FACTOR= 1.0000E+00

Appendix 14. Correction Formula for Adjusting Chromatogram Peak Areas for Contamination.

Fatty Acid Calculations (with corrections for blank)

(i) On the data sheet, record the peak areas of 15:0, 16:0, 18:0, 18:1 for a set of blanks (e.g. 3 days, one subject). Average the 3 values for each peak area.

(ii) On the sample chromatogram, correct the 16:0, 18:0, 18:1 "Area %" values:

For PC:

$$A = \frac{\text{peak area FA (blank)}}{\text{peak area 15:0 (blank)}} \times \frac{\text{peak area 15:0 (sample)}}{2}$$

For PE or PPE:

$$A = \frac{\text{peak area FA (blank)}}{\text{peak area 15:0 (blank)}} \times \text{peak area 15:0 (sample)}$$

$$\text{Corrected "AREA \%" for FA} = \frac{\text{peak area FA (sample)} - A}{\text{total chromatogram area}} \times 100$$

Replace the "AREA %" with corrected values.

(iii) Add up "AREA %" for peaks of interest.

(iv) Calculate "AREA %" out of 100 for each fatty acid.

Appendix 15. Samples of Data Analysis.
A. Comparison of Means of Mixed Fat Diet and Experimental Diets Using the Paired T-Test.

```
  1. // JOB
  2. // EXEC SAS
  3. //SYSIN DD *
  4. DATA PHO2;
  5. INPUT FRAC $ 1-3 GROUP $ 5 D716W0 10-14 D718W0 16-20
     D718W1 22-26
  6. D718W2 28-32 D718W3 34-37 D720W3 39-42 D720W4 44-48
     D720W5 50-53
  7. D722W4 55-58 D722W5 60-63 D722W6 65-69
  8. #2 D2516W0 10-14 D2518W0 16-20 D2518W1 22-26 D2518W2
     28-32 D2518W3
  9. 34-37 D2520W3 39-42 D2520W4 44-48 D2520W5 50-53 D2522W4
     55-58 D2522W5
 10. 60-63 D2522W6 65-69;
 11. D7N6 = D720W3 + D720W4 + D722W4;
 12. D7N3 = D720W5 + D722W5 + D722W6;
 13. D25N6 = D2520W3 + D2520W4 + D2522W4;
 14. D25N3 = D2520W5 + D2522W5 + D2522W6;
 15. DIF16W0 = D716W0 - D2516W0;
 16. DIF18W0 = D718W0 - D2518W0;
 17. DIF18W1 = D718W1 - D2518W1;
 18. DIF18W2 = D718W2 - D2518W2;
 19. DIF18W3 = D718W3 - D2518W3;
 20. DIF20W3 = D720W3 - D2520W3;
 21. DIF20W4 = D720W4 - D2520W4;
 22. DIF20W5 = D720W5 - D2520W5;
 23. DIF22W4 = D722W4 - D2522W4;
 24. DIF22W5 = D722W5 - D2522W5;
 25. DIF22W6 = D722W6 - D2522W6;
 26. DIFN6 = D7N6 - D25N6;
 27. DIFN3 = D7N3 - D25N3;
 28. CARDS;
 29. PC A  7 29.99 13.21 14.65 27.55 0.11 2.58  8.00 0.42
     0.27 0.66  1.65
118. PPE D 25  0.83  0.16  3.15 18.22 1.14 0.98 46.86 4.52
     1.75 5.79 16.27
119. PROC PRINT DATA=PHO2;
120. PROC SORT;
121. BY FRAC GROUP;
122. PROC MEANS DATA=PHO2 N MEAN VAR STD STDERR T;
123. BY FRAC GROUP;
124. VAR D716W0 D718W0 D718W1 D718W2 D718W3 D720W3 D720W4
     D720W5 D722W4
125. D722W5 D722W6 D2516W0 D2518W0 D2518W1 D2518W2 D2518W3
     D2520W3 D2520W4
126. D2520W5 D2522W4 D2522W5 D2522W6 D7N6 D7N3 D25N6 D25N3
     DIF16W0 DIF18W0
127. DIF18W1 DIF18W2 DIF18W3 DIF20W3 DIF20W4 DIF20W5 DIF22W4
```

Appendix 15 (cont'd).
B. Results of the Paired T-Test.

FRAC=PC GROUP=A

VARIABLE	N	MEAN	VARIANCE	STANDARD DEVIATION	STD ERROR OF MEAN	T
DIF16W0	4	3.51500000	30.85096667	5.55436465	2.77718233	1.27
DIF18W0	4	-0.23500000	0.66310000	0.81430952	0.40715476	-0.58
DIF18W1	4	-2.23250000	3.63349167	1.90617199	0.95308600	-2.34
DIF18W2	4	0.61750000	2.02909167	1.42446189	0.71223094	0.87
DIF18W3	4	-0.29000000	0.02000000	0.14142136	0.07071068	-4.10
DIF20W3	4	0.49750000	0.24089167	0.49080716	0.24540358	2.03
DIF20W4	4	-0.53250000	2.17922500	1.47621983	0.73810992	-0.72
DIF20W5	4	-0.60750000	0.13262500	0.36417715	0.18208858	-3.34
DIF22W4	4	0.05250000	0.00302500	0.05500000	0.02750000	1.91
DIF22W5	4	-0.12750000	0.06522500	0.25539186	0.12769593	-1.00
DIF22W6	4	-0.20750000	0.47715833	0.69076648	0.34538324	-0.60
DIFN6	4	0.01750000	3.79075833	1.94698699	0.97349349	0.02
DIFN3	4	-0.94250000	1.63649167	1.27925434	0.63962717	-1.47

Appendix 15 (cont'd).
C. Comparison of Mean Differences of Experimental Diets.

```
1. // JOB
2. // EXEC SAS
3. //SYSIN DD *
4. DATA CHO2;
5. INPUT GROUP 1 D25TG 6-9 D25TC 11-14 D25HDL 16-19 D25LDL
   21-24 D25VLDL
6. 26-29
7. #2 D43TG 6-9 D43TC 11-14 D43HDL 16-19 D43LDL 21-24
   D43VLDL   26-29;
8. DIFTG = D25TG - D43TG;
9. DIFTC = D25TC - D43TC;
10. DIFHDL = D25HDL - D43HDL;
11. DIFLDL = D25LDL - D43LDL;
12. DIFVLDL = D25VLDL - D43VLDL;
13. CARDS;
14. 1 25 1.06 3.66 1.01 2.35 0.30
43. 4 43 1.19 5.73 0.86 4.62 0.25
44. PROC PRINT DATA=CHO2;
45. PROC SORT;
46. BY GROUP;
47. PROC GLM;
48. CLASS GROUP;
49. MODEL DIFTG=GROUP;
50. MEANS GROUP/T;
51. LSMEANS GROUP/PDIFF;
```

Appendix 15 (cont'd).
D. Results of the General Linear Models Procedure.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: DIFTG

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	3	0.74331833	0.24777278	3.83	0.0423	0.510839	359.9651
ERROR	11	0.71177500	0.06470682		ROOT MSE		DIFTG MEAN
CORRECTED TOTAL	14	1.45509333			0.25437535		0.07066667

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE III SS	F VALUE	PR > F
GROUP	3	0.74331833	3.83	0.0423	3	0.74331833	3.83	0.0423

Appendix 15 (cont'd).
D. Results of the General Linear Models Procedure (cont'd).

GENERAL LINEAR MODELS PROCEDURE

T TESTS (LSD) FOR VARIABLE: DIFTG
NOTE: THIS TEST CONTROLS THE TYPE I COMPARISONWISE ERROR RATE, NOT THE EXPERIMENTWISE ERROR RATE.

ALPHA=0.05 CONFIDENCE=0.95 DF=11 MSE=.0647068
CRITICAL VALUE OF T=2.20099

COMPARISONS SIGNIFICANT AT THE 0.05 LEVEL ARE INDICATED BY '***'

GROUP COMPARISON		LOWER CONFIDENCE LIMIT	DIFFERENCE BETWEEN MEANS	UPPER CONFIDENCE LIMIT	
1	- 3	0.0491	0.4450	0.8409	***
1	- 2	0.0774	0.5050	0.9326	***
1	- 4	0.1466	0.5425	0.9384	***
3	- 1	-0.8409	-0.4450	-0.0491	***
3	- 2	-0.3676	0.0600	0.4876	
3	- 4	-0.2984	0.0975	0.4934	
2	- 1	-0.9326	-0.5050	-0.0774	***
2	- 3	-0.4876	-0.0600	0.3676	
2	- 4	-0.3901	0.0375	0.4651	
4	- 1	-0.9384	-0.5425	-0.1466	***
4	- 3	-0.4934	-0.0975	0.2984	
4	- 2	-0.4651	-0.0375	0.3901	

GENERAL LINEAR MODELS PROCEDURE

LEAST SQUARES MEANS

GROUP	DIFTG LSMEAN	PROB > |T| H0: LSMEAN(I)=LSMEAN(J) I/J	1	2	3	4
1	0.43500000	1	.	0.0247	0.0309	0.0117
2	-0.07000000	2	0.0247	.	0.7632	0.8505
3	-0.01000000	3	0.0309	0.7632	.	0.5986
4	-0.10750000	4	0.0117	0.8505	0.5986	.

NOTE: TO ENSURE OVERALL PROTECTION LEVEL, ONLY PROBABILITIES ASSOCIATED WITH PRE-PLANNED COMPARISONS SHOULD BE USED.

Appendix 15 (cont'd).
E. Comparison of Means of Experimental Diets. (Results of this analysis are similar to those presented in Appendix 15.D.)

```
  1. // JOB
  2. // EXEC SAS
  3. //SYSIN DD *
  4. DATA PHO1B;
  5. INPUT GROUP $ 1 DIET $ 3-5 FRAC $ 7-9 DAY 11-12 FA20W3
     14-17 FA20W4
  6. 19-23 FA20W5 25-28 FA22W4 30-33 FA22W5 35-38 FA22W6
     40-44;
  7. N6 = FA20W3 + FA20W4 + FA22W4;
  8. N3 = FA20W5 + FA22W5 + FA22W6;
  9. CARDS;
 10. A MIX  PC  7 2.58  8.00 0.42 0.27 0.66  1.65
144. D SO  PPE 43 1.52 50.52 1.53 1.27 2.22  9.04
145. PROC PRINT DATA=PHO1B;
146. VAR GROUP DIET FRAC DAY FA20W3 FA20W4 FA20W5 FA22W4
     FA22W5 FA22W6 N6 N3;
147. PROC SORT DATA=PHO1B;
148. BY GROUP DIET FRAC DAY;
149. PROC MEANS DATA=PHO1B;
150. BY GROUP DIET FRAC DAY;
151. VAR FA20W3 FA20W4 FA20W5 FA22W4 FA22W5 FA22W6 N6 N3;
152. DATA PHO2B;
153. SET PHO1B;
154. IF DAY=25 OR DAY=43;
155. PROC SORT DATA=PHO2B;
156. BY FRAC DAY;
157. PROC GLM DATA=PHO2B;
158. BY FRAC DAY;
159. CLASS DIET;
160. MODEL FA20W3=DIET;
161. MEANS DIET/LSD;
162. LSMEANS DIET/PDIFF;
```

Appendix 16. Plasma Lipid and Lipoprotein Levels for Individual Subjects During the Study.

Group / Subject	Total Cholesterol (mmol/L)				
	Day: 1	7	25	43	49
S/O -- SOY					
1	6.18	5.79	5.05	4.84	5.74
7	7.12	6.33	5.21	5.52	6.26
12	5.55	4.92	4.73	4.59	4.24
SOY -- CAN					
2	6.64	7.06	5.82	5.54	6.45
3	6.80	5.91	4.36	4.40	5.27
11	5.60	5.94	5.46	5.15	5.98
15	6.08	6.39	5.01	4.64	5.22
CAN -- S/O/F					
4	4.78	4.34	3.66	3.48	3.60
8	7.70	6.61	5.51	5.71	5.61
10	6.37	5.32	4.53	5.10	5.30
14	5.63	5.32	4.18	4.58	5.39
S/O/F -- S/O					
5	6.44	6.01	5.32	4.77	5.55
6	5.90	4.56	4.76	4.43	4.56
9	6.69	6.07	5.44	5.22	5.69
13	5.37	6.95	5.49	5.73	6.07

cont'd...

Appendix 16 (cont'd).

Group / Subject	Low Density Lipoprotein-Cholesterol (mmol/L)				
	Day: 1	7	25	43	49
S/O -- SOY					
1	5.02	4.58	4.05	3.71	4.29
7	4.94	4.60	3.79	3.97	4.32
12	4.24	3.59	3.49	3.58	2.59
SOY -- CAN					
2	5.16	5.22	4.58	3.95	4.77
3	3.73	4.00	3.31	3.29	3.85
11	4.13	4.55	4.03	3.68	4.27
15	4.25	4.89	3.97	3.66	3.88
CAN -- S/O/F					
4	3.40	2.87	2.35	2.30	2.20
8	5.65	4.51	3.91	4.39	4.19
10	4.54	3.82	3.07	3.83	3.61
14	4.12	4.33	2.98	3.39	3.74
S/O/F -- S/O					
5	5.09	4.83	3.86	3.77	4.22
6	4.72	3.38	3.53	3.28	3.62
9	4.42	4.36	3.87	3.61	4.09
13	4.38	5.59	4.51	4.62	4.69

cont'd...

Appendix 16 (cont'd).

Group / Subject	High Density Lipoprotein-Cholesterol (mmol/L)				
	Day: 1	7	25	43	49
S/O -- SOY					
1	0.86	1.10	1.00	0.87	0.92
7	0.96	1.17	1.05	0.92	1.03
12	1.24	1.18	1.11	1.00	0.94
SOY -- CAN					
2	0.83	0.87	0.94	0.77	0.97
3	0.59	1.02	0.85	0.75	0.87
11	1.32	1.39	1.43	1.46	1.46
15	1.05	1.16	1.03	0.98	0.99
CAN -- S/O/F					
4	1.04	1.27	1.01	0.81	0.90
8	1.08	1.06	0.92	0.94	1.01
10	1.09	0.94	0.85	0.80	0.69
14	0.94	0.86	0.82	0.74	0.88
S/O/F -- S/O					
5	1.12	1.13	1.13	0.90	1.05
6	0.88	1.05	0.75	0.76	0.85
9	1.12	1.11	1.10	1.03	1.04
13	0.81	1.05	0.91	0.86	0.86

cont'd...

Appendix 16 (cont'd).

Group / Subject	Very Low Density Lipoprotein-Cholesterol (mmol/L)				
	Day: 1	7	25	43	49
S/O -- SOY					
1	0.30	0.11	0.00	0.26	0.53
7	1.22	0.56	0.37	0.63	0.91
12	0.07	0.15	0.13	0.01	0.71
SOY -- CAN					
2	0.65	0.97	0.30	0.82	0.71
3	2.48	0.89	0.20	0.36	0.55
11	0.15	0.00	0.00	0.00	0.25
15	0.78	0.34	0.01	0.00	0.35
CAN -- S/O/F					
4	0.34	0.20	0.30	0.37	0.50
8	0.97	1.04	0.68	0.38	0.41
10	0.74	0.56	0.61	0.47	1.00
14	0.57	0.13	0.38	0.45	0.77
S/O/F -- S/O					
5	0.23	0.05	0.33	0.10	0.28
6	0.30	0.13	0.48	0.39	0.09
9	1.15	0.60	0.47	0.58	0.56
13	0.18	0.31	0.07	0.25	0.52

cont'd...

Appendix 16 (cont'd).

Group / Subject	Triglyceride (mmol/L) Day: 1	7	25	43	49
S/O -- SOY					
1	0.95	0.87	0.89	0.86	1.21
7	2.58	1.30	1.17	1.52	1.83
12	1.11	0.91	1.14	1.03	1.45
SOY -- CAN					
2	3.19	2.11	1.45	1.50	2.06
3	6.58	1.51	0.74	0.90	1.14
11	0.68	0.65	0.55	0.64	0.69
15	2.15	1.06	0.85	0.59	1.13
CAN -- S/O/F					
4	0.96	0.75	1.06	0.91	0.99
8	1.46	1.93	1.68	1.07	1.27
10	1.74	1.47	1.96	1.11	2.51
14	1.37	1.31	1.04	0.91	1.62
S/O/F -- S/O					
5	1.22	0.68	0.61	0.82	1.06
6	1.18	0.73	1.13	1.34	0.98
9	2.76	1.98	1.79	1.61	1.96
13	1.16	1.07	1.00	1.19	1.44

Appendix 17. The Fatty Acid Composition of Plasma Phosphatidylcholine for Individual Subjects During the Study.

Group / Subject	Fatty Acid[1] (% of total)					
	16:0			16:1		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	36.91	40.08	34.60	0.37	0.79	0.26
7	32.85	26.81	35.23	0.46	0.50	0.43
12	40.79	40.58	32.87	0.61	0.57	0.36
SOY -- CAN						
2	28.56	31.42	24.06	0.43	0.30	0.37
3	33.86	33.10	25.00	0.38	0.22	0.33
11	29.19	34.57	36.42	0.32	0.26	0.45
15	27.28	32.39	33.79	0.47	0.25	0.43
CAN -- S/O/F						
4	29.99	26.28	28.32	0.39	0.42	0.41
8	30.50	29.10	30.44	0.39	0.41	0.37
10	32.02	34.11	36.88	0.50	0.48	0.42
14	38.08	27.04	33.85	0.39	0.34	0.30
S/O/F -- S/O						
5	34.28	37.67	32.51	0.86	0.77	0.50
6	37.35	37.70	33.20	0.37	0.74	0.49
9	30.30	34.00	36.14	0.81	0.71	0.91
13	29.85	28.16	34.16	0.25	0.21	0.32

[1] Carbon chain length : number of double bonds

cont'd...

Appendix 17 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	18:0			18:1		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	14.64	19.57	16.16	12.61	11.12	7.13
7	14.99	14.78	14.14	14.31	16.46	9.20
12	12.02	11.48	13.86	12.95	14.84	8.53
SOY -- CAN						
2	14.38	14.28	14.32	15.24	9.84	18.29
3	13.23	13.66	13.40	14.49	8.16	17.58
11	13.01	12.68	10.39	14.51	8.44	15.91
15	14.62	15.88	12.13	13.97	7.11	15.60
CAN -- S/O/F						
4	13.21	13.70	14.24	14.65	17.10	11.80
8	14.71	14.83	13.90	13.33	13.66	12.05
10	12.95	12.14	12.70	14.72	16.09	11.66
14	12.22	13.36	14.11	12.70	17.48	10.35
S/O/F -- S/O						
5	18.70	19.45	13.05	12.55	9.04	16.99
6	11.96	16.27	11.52	14.18	9.99	16.59
9	14.14	13.74	12.44	14.60	11.60	14.88
13	15.16	15.81	13.41	12.04	8.88	14.15

cont'd...

Appendix 17 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	18:2			18:3		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	23.13	13.93	28.28	0.13	0.00	0.35
7	22.37	22.67	27.94	0.10	0.07	0.24
12	23.79	22.72	30.60	0.13	0.07	0.23
SOY -- CAN						
2	22.89	28.82	22.98	0.10	0.23	0.48
3	25.45	32.49	27.09	0.10	0.28	0.58
11	29.34	34.48	27.53	0.14	0.31	0.42
15	26.61	32.10	27.65	0.13	0.26	0.42
CAN -- S/O/F						
4	27.55	25.89	31.44	0.11	0.48	0.47
8	25.17	23.16	26.91	0.13	0.38	0.44
10	25.27	26.09	28.00	0.15	0.26	0.64
14	26.27	26.65	30.65	0.10	0.53	0.85
S/O/F -- S/O						
5	20.30	21.01	26.15	0.17	0.88	0.08
6	28.16	25.80	29.66	0.09	0.85	0.09
9	23.70	27.07	23.28	0.14	0.85	0.12
13	24.23	30.16	23.29	0.09	0.68	0.06

cont'd...

Appendix 17 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	20:0			20:1		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	0.03	0.58	0.04	0.14	0.51	0.11
7	0.04	0.09	0.05	0.17	0.31	0.13
12	0.02	0.04	0.07	0.09	0.12	0.11
SOY -- CAN						
2	0.05	0.07	0.18	0.21	0.17	0.70
3	0.04	0.09	0.21	0.14	0.15	0.68
11	0.05	0.07	0.10	0.23	0.15	0.37
15	0.05	0.07	0.08	0.23	0.13	0.34
CAN -- S/O/F						
4	0.04	0.16	0.08	0.20	0.62	0.23
8	0.04	0.10	0.06	0.16	0.38	0.19
10	0.04	0.08	0.04	0.22	0.32	0.14
14	0.04	0.19	0.09	0.13	0.61	0.19
S/O/F -- S/O						
5	0.10	0.70	0.07	0.20	0.26	0.27
6	0.05	0.22	0.08	0.14	0.22	0.29
9	0.04	0.05	0.03	0.18	0.15	0.17
13	0.05	0.08	0.07	0.17	0.20	0.23

cont'd...

Appendix 17 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	20:2			20:3		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	0.31	0.26	0.34	3.24	2.98	2.74
7	0.29	0.33	0.32	3.04	3.47	1.64
12	0.23	0.20	0.35	2.49	2.49	2.60
SOY -- CAN						
2	0.34	0.39	0.44	3.79	1.82	2.70
3	0.28	0.37	0.39	2.81	1.32	1.95
11	0.38	0.47	0.28	2.47	1.11	1.23
15	0.43	0.38	0.24	4.16	2.07	1.45
CAN -- S/O/F						
4	0.29	0.41	0.33	2.58	2.49	2.00
8	0.33	0.36	0.33	2.97	2.59	2.43
10	0.33	0.28	0.26	4.53	3.32	3.09
14	0.22	0.36	0.28	2.21	1.90	1.27
S/O/F -- S/O						
5	0.29	0.41	0.29	2.43	1.26	2.24
6	0.24	0.37	0.37	1.39	1.29	2.44
9	0.40	0.29	0.28	3.75	1.89	2.40
13	0.39	0.36	0.29	2.84	1.68	2.41

cont'd...

Appendix 17 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	20:4			20:5		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	6.58	7.47	7.73	0.46	0.28	0.56
7	8.73	11.50	8.53	0.40	0.24	0.28
12	5.46	5.91	7.91	0.36	0.19	0.43
SOY -- CAN						
2	10.17	10.04	11.10	0.55	0.35	1.26
3	7.20	8.23	9.07	0.36	0.35	1.23
11	7.12	5.76	5.20	0.43	0.21	0.51
15	8.68	7.59	6.64	0.52	0.33	0.53
CAN -- S/O/F						
4	8.00	8.74	7.36	0.42	1.06	0.74
8	8.71	9.66	9.16	0.47	1.43	0.60
10	6.41	4.88	4.47	0.37	0.47	0.42
14	6.26	8.23	5.96	0.32	1.05	0.88
S/O/F -- S/O						
5	6.97	5.33	6.68	0.39	0.71	0.16
6	4.75	3.91	4.06	0.18	0.30	0.11
9	8.96	6.96	7.73	0.50	0.83	0.27
13	9.43	9.20	9.36	0.62	1.08	0.20

cont'd...

Appendix 17 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	22:4			22:5		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	0.17	0.24	0.14	0.32	0.34	0.42
7	0.24	0.34	0.13	0.51	0.47	0.43
12	0.06	0.02	0.15	0.30	0.17	0.64
SOY -- CAN						
2	0.32	0.16	0.19	1.01	0.63	1.04
3	0.15	0.04	0.14	0.50	0.49	0.83
11	0.27	0.11	0.07	0.84	0.40	0.37
15	0.40	0.07	0.03	0.87	0.45	0.10
CAN -- S/O/F						
4	0.27	0.19	0.19	0.66	0.81	0.82
8	0.27	0.19	0.23	0.59	0.81	0.76
10	0.21	0.13	0.03	0.61	0.38	0.41
14	0.08	0.11	0.02	0.37	0.74	0.52
S/O/F -- S/O						
5	0.21	0.40	0.06	0.56	0.59	0.23
6	0.05	0.08	0.06	0.16	0.29	0.16
9	0.36	0.07	0.17	0.68	0.64	0.34
13	0.22	0.16	0.14	0.71	0.75	0.27

cont'd...

Appendix 17 (cont'd).

Group / Subject	Fatty Acid (% of total) 22:6		
	Day: 7	25	43
S/O -- SOY			
1	0.96	1.85	1.14
7	1.52	1.98	1.31
12	0.70	0.59	1.28
SOY -- CAN			
2	1.97	1.46	1.90
3	1.01	1.05	1.51
11	1.70	0.97	0.76
15	1.58	0.92	0.55
CAN -- S/O/F			
4	1.65	1.65	1.57
8	2.23	2.96	2.12
10	1.66	0.98	0.83
14	0.63	1.41	0.70
S/O/F -- S/O			
5	2.00	1.52	0.71
6	0.94	1.97	0.88
9	1.43	1.17	0.84
13	3.95	2.59	1.64

Appendix 18. The Fatty Acid Composition of Plasma Phosphatidylethanolamine for Individual Subjects During the Study.

Group / Subject	Fatty Acid[1] (% of total) 16:0			16:1		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	14.69	13.82	13.83	0.45	0.37	0.36
7	15.65	12.70	17.06	0.58	0.43	0.46
12	22.58	20.47	13.89	0.47	0.24	0.17
SOY -- CAN						
2	21.42	12.22	14.66	0.35	0.35	0.28
3	13.88	11.90	10.91	0.45	0.39	0.45
11	23.70	17.87	15.59	0.29	0.20	0.22
15	10.95	10.89	12.68	0.48	0.24	0.37
CAN -- S/O/F						
4	15.90	11.22	11.14	0.18	0.56	0.21
8	14.21	14.53	14.92	0.35	0.34	0.48
10	16.31	16.05	18.65	0.20	0.18	0.26
14	18.95	13.13	12.26	0.23	0.15	0.13
S/O/F -- S/O						
5	12.82	10.22	11.91	0.41	0.39	0.38
6	16.48	12.06	12.32	0.47	0.35	0.35
9	19.23	16.11	19.84	0.59	0.35	0.51
13	23.00	16.79	16.66	0.22	0.15	0.16

[1] Carbon chain length : number of double bonds

cont'd...

Appendix 18 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	18:0			18:1		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	32.12	31.58	35.53	11.59	14.77	8.66
7	30.07	29.39	34.09	12.57	15.51	10.81
12	29.38	28.56	31.30	12.43	18.16	9.88
SOY -- CAN						
2	32.21	33.41	30.56	13.67	12.00	19.80
3	33.55	34.02	30.87	11.05	10.09	19.73
11	26.74	26.45	24.06	12.39	11.55	19.49
15	28.15	33.56	26.39	14.20	9.70	20.77
CAN -- S/O/F						
4	28.30	28.26	29.65	12.23	15.92	11.66
8	32.74	33.80	33.33	10.54	10.56	9.60
10	28.35	29.27	30.22	12.19	15.72	11.40
14	31.44	30.42	34.74	11.31	17.25	11.54
S/O/F -- S/O						
5	33.19	35.75	29.86	13.74	13.70	20.39
6	32.34	32.80	29.74	10.80	10.59	15.78
9	31.59	32.84	28.70	12.57	12.23	16.59
13	31.26	31.43	28.73	8.31	9.88	13.70

cont'd...

Appendix 18 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	18:2			18:3		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	10.49	10.01	12.83	0.16	0.12	0.41
7	9.27	8.56	13.24	0.14	0.10	0.33
12	14.05	13.08	15.90	0.19	0.12	0.34
SOY -- CAN						
2	12.18	12.74	10.19	0.12	0.29	0.53
3	9.29	13.97	11.16	0.28	0.50	0.68
11	15.81	20.16	13.30	0.17	0.53	0.54
15	13.82	17.55	14.83	0.20	0.44	0.68
CAN -- S/O/F						
4	13.96	10.28	14.94	0.14	0.60	0.52
8	11.37	9.53	10.69	0.17	0.38	0.39
10	14.20	15.83	17.98	0.18	0.34	0.77
14	13.34	11.86	16.48	0.14	0.58	1.14
S/O/F -- S/O						
5	11.31	13.84	12.24	0.14	0.94	0.17
6	11.73	18.18	16.14	0.15	0.85	0.12
9	14.37	15.34	15.58	0.13	0.87	0.15
13	11.32	15.72	12.20	0.12	0.99	0.00

cont'd...

Appendix 18 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	20:0			20:1		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	0.23	0.38	0.39	0.26	0.43	0.30
7	0.16	0.25	0.17	0.32	0.52	0.26
12	0.14	0.19	0.24	0.17	0.35	0.26
SOY -- CAN						
2	0.08	0.25	0.25	0.17	0.33	0.67
3	0.25	0.40	0.47	0.36	0.28	0.80
11	0.00	0.30	0.34	0.19	0.24	0.71
15	0.29	0.32	0.28	0.34	0.17	0.58
CAN -- S/O/F						
4	0.14	0.31	0.31	0.38	0.80	0.41
8	0.17	0.21	0.24	0.25	0.46	0.30
10	0.18	0.15	0.13	0.33	0.38	0.25
14	0.15	0.45	0.30	0.17	0.76	0.25
S/O/F -- S/O						
5	0.29	0.57	0.29	0.33	0.51	0.53
6	0.30	0.30	0.26	0.37	0.42	0.62
9	0.14	0.27	0.12	0.25	0.25	0.22
13	0.12	0.17	0.20	0.17	0.23	0.33

cont'd...

Appendix 18 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	20:2			20:3		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	0.10	0.15	0.30	1.51	1.78	1.23
7	0.10	0.19	0.28	1.27	1.37	0.78
12	0.00	0.11	0.25	1.09	1.21	1.19
SOY -- CAN						
2	0.07	0.39	0.28	1.05	0.95	0.96
3	0.10	0.36	0.00	1.12	0.73	0.88
11	0.00	0.35	0.30	0.86	0.78	0.99
15	0.14	0.32	0.17	1.55	0.96	0.85
CAN -- S/O/F						
4	0.09	0.28	0.26	1.13	1.22	1.13
8	0.10	0.15	0.15	0.99	0.90	0.87
10	0.10	0.14	0.09	2.04	1.85	1.50
14	0.00	0.00	0.25	1.15	1.01	0.77
S/O/F -- S/O						
5	0.13	0.33	0.24	1.26	0.88	1.32
6	0.13	0.30	0.30	0.74	0.96	1.61
9	0.00	0.17	0.00	1.11	0.78	1.07
13	0.00	0.15	0.17	0.75	0.59	1.08

cont'd...

Appendix 18 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	20:4			20:5		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	17.32	17.98	16.87	0.66	0.38	0.53
7	20.02	21.67	16.77	0.46	0.22	0.30
12	14.31	13.79	18.50	0.61	0.25	0.38
SOY -- CAN						
2	14.67	18.25	14.89	0.40	0.42	1.00
3	20.00	19.32	17.38	0.52	0.43	1.06
11	15.78	16.17	17.11	0.45	0.39	0.88
15	20.36	19.31	16.48	0.67	0.42	0.77
CAN -- S/O/F						
4	18.96	18.04	19.32	0.56	1.39	1.04
8	17.13	16.23	18.10	0.52	1.15	0.58
10	17.42	13.64	13.25	0.51	0.71	0.60
14	17.55	18.48	16.74	0.45	1.26	1.09
S/O/F -- S/O						
5	19.59	16.25	17.39	0.51	0.92	0.27
6	17.20	14.66	14.48	0.35	0.54	0.26
9	16.11	15.50	14.50	0.38	0.73	0.25
13	15.73	16.21	19.55	0.63	0.98	0.28

cont'd...

Appendix 18 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	22:4			22:5		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	0.68	0.94	0.62	1.45	1.05	1.49
7	0.61	0.63	0.32	1.25	0.82	0.75
12	0.42	0.22	0.58	1.11	0.62	1.42
SOY -- CAN						
2	0.18	0.35	0.00	0.55	1.31	0.87
3	0.53	0.48	0.00	1.38	1.39	1.13
11	0.09	0.37	0.13	0.95	1.27	1.63
15	1.12	0.52	0.06	2.91	1.69	1.48
CAN -- S/O/F						
4	0.52	0.28	0.55	1.36	1.51	2.13
8	0.47	0.25	0.43	0.99	1.02	1.20
10	0.52	0.30	0.31	1.63	1.15	1.16
14	0.30	0.00	0.24	1.27	1.30	1.45
S/O/F -- S/O						
5	0.71	0.43	0.52	1.39	1.63	0.93
6	0.47	0.33	0.38	0.79	0.87	0.68
9	0.55	0.30	0.23	0.85	1.31	0.60
13	0.25	0.19	0.58	1.04	1.24	0.85

cont'd...

Appendix 18 (cont'd).

Group / Subject	Fatty Acid (% of total) 22:6		
Day:	7	25	43
S/O -- SOY			
1	8.29	6.24	6.67
7	7.50	7.65	4.38
12	3.05	2.63	5.69
SOY -- CAN			
2	2.87	6.74	5.06
3	7.24	5.73	4.48
11	2.58	3.37	4.71
15	4.83	3.91	3.60
CAN -- S/O/F			
4	6.16	9.33	6.74
8	10.00	10.47	8.71
10	5.86	4.28	3.45
14	3.54	3.36	2.63
S/O/F -- S/O			
5	4.19	3.65	3.56
6	7.67	6.79	6.95
9	2.12	2.96	1.63
13	7.08	5.29	5.50

Appendix 19. The Fatty Acid Composition of Plasma Alkenylacyl Ethanolamine Phosphoglyceride for Individual Subjects During the Study.

Group / Subject	Fatty Acid[1] (% of total)					
	16:0			16:1		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	10.82	1.13	2.79	0.10	0.21	0.18
7	0.44	1.55	0.00	0.24	0.31	0.47
12	3.29	7.31	0.56	0.68	0.53	0.13
SOY -- CAN						
2	0.96	0.01	1.20	0.41	0.00	0.11
3	0.00	0.00	0.00	0.06	0.15	0.12
11	2.77	3.94	2.72	0.57	0.31	0.33
15	0.00	0.32	0.00	0.19	0.23	1.23
CAN -- S/O/F						
4	0.00	0.00	0.00	0.30	0.98	0.47
8	0.43	0.00	1.02	0.27	0.29	0.00
10	0.24	0.00	0.75	0.21	0.00	0.24
14	1.34	3.11	0.71	0.32	0.16	0.32
S/O/F -- S/O						
5	1.09	1.13	1.50	0.23	0.00	0.16
6	0.25	0.00	0.00	0.17	0.12	0.07
9	1.19	1.93	4.01	0.40	0.56	0.66
13	2.78	0.83	2.91	0.50	0.14	0.39

[1] Carbon chain length : number of double bonds

cont'd...

Appendix 19 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	18:0			18:1		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	0.82	0.12	0.28	3.73	7.26	2.88
7	0.19	0.30	0.00	4.85	5.49	3.29
12	1.05	2.60	0.08	6.08	9.91	2.20
SOY -- CAN						
2	0.00	0.00	0.00	6.60	4.16	7.09
3	0.00	0.00	0.00	1.97	1.65	4.55
11	0.00	0.18	0.04	7.93	5.17	8.54
15	0.00	0.00	0.00	4.03	3.60	9.36
CAN -- S/O/F						
4	0.00	0.00	0.00	6.64	5.13	4.26
8	0.00	0.00	0.00	3.17	4.90	2.00
10	0.00	0.00	0.00	4.82	7.72	6.30
14	0.00	0.00	0.00	5.90	8.09	5.02
S/O/F -- S/O						
5	0.26	0.00	0.07	4.49	2.98	5.58
6	0.61	0.33	0.00	4.55	4.07	5.82
9	0.43	0.47	1.35	4.97	5.09	8.27
13	1.27	0.16	0.39	5.79	3.15	8.35

cont'd...

Appendix 19 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	18:2			18:3		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	10.50	14.36	14.60	0.00	0.00	0.51
7	11.67	10.70	18.99	0.75	0.48	0.54
12	19.01	21.04	12.12	0.20	0.17	0.35
SOY -- CAN						
2	22.48	27.38	15.78	0.23	0.64	0.69
3	7.28	14.97	11.46	0.34	1.47	0.80
11	29.01	32.63	22.40	0.36	0.66	0.62
15	15.03	27.83	25.03	0.22	0.57	0.81
CAN -- S/O/F						
4	18.62	12.23	22.07	0.29	0.98	1.60
8	11.22	14.81	13.13	0.47	1.34	2.42
10	16.73	20.01	21.14	0.21	0.40	1.00
14	18.97	18.12	23.69	0.26	0.77	1.70
S/O/F -- S/O						
5	15.31	15.44	14.56	0.25	1.02	0.14
6	15.39	21.41	20.21	0.23	1.16	0.00
9	13.92	18.47	16.61	0.15	0.99	0.22
13	21.33	18.22	21.57	0.20	1.14	0.17

cont'd...

Appendix 19 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	20:0			20:1		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	1.42	0.00	0.14	0.00	0.15	0.07
7	0.00	0.00	0.00	0.00	0.12	0.00
12	0.00	0.00	0.08	0.08	0.13	0.08
SOY -- CAN						
2	0.00	0.00	0.00	0.00	0.00	0.24
3	0.00	0.15	0.18	0.08	0.00	0.21
11	0.00	0.00	0.00	0.00	0.08	0.27
15	0.06	0.00	0.00	0.19	0.00	0.26
CAN -- S/O/F						
4	0.00	0.32	0.00	0.14	0.29	0.00
8	0.18	0.00	1.85	0.12	0.00	0.00
10	0.00	0.00	0.00	0.10	0.00	0.00
14	0.00	0.00	0.00	0.00	0.24	0.00
S/O/F -- S/O						
5	0.06	0.13	0.17	0.11	0.00	0.12
6	0.13	0.12	0.00	0.09	0.14	0.00
9	0.00	0.00	0.00	0.10	0.03	0.19
13	0.00	0.02	0.00	0.15	0.10	0.11

cont'd...

Appendix 19 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	20:2			20:3		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	0.00	0.00	0.00	2.21	2.44	1.46
7	0.00	0.00	0.00	2.08	1.66	0.92
12	0.00	0.00	0.07	2.95	2.78	1.69
SOY -- CAN						
2	0.00	0.00	0.00	3.13	1.32	1.82
3	0.00	0.00	0.00	2.28	1.04	1.49
11	0.00	0.13	0.12	2.06	1.03	1.32
15	0.00	0.00	0.00	3.57	1.21	1.32
CAN -- S/O/F						
4	0.00	0.00	0.00	2.33	1.23	1.09
8	0.00	0.00	0.00	1.77	0.47	1.23
10	0.00	0.00	0.00	4.06	3.17	3.59
14	0.00	0.00	0.00	2.81	1.75	1.35
S/O/F -- S/O						
5	0.00	0.00	0.00	2.88	1.28	2.08
6	0.00	0.07	0.00	1.85	1.40	2.20
9	0.00	0.00	0.00	2.25	1.27	1.75
13	0.00	0.07	0.00	1.87	0.98	1.52

cont'd...

Appendix 19 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	20:4			20:5		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	42.08	51.56	45.80	2.73	1.75	2.33
7	56.43	56.99	58.96	2.65	1.30	1.52
12	44.40	42.76	49.48	3.45	1.83	2.51
SOY -- CAN						
2	52.26	55.83	53.65	3.12	1.86	4.96
3	54.72	50.66	50.73	3.24	1.75	5.30
11	42.31	41.89	46.54	3.17	1.70	3.56
15	51.39	49.52	48.09	3.69	2.17	3.86
CAN -- S/O/F						
4	47.68	46.48	47.66	2.95	4.14	3.45
8	51.31	53.75	55.13	3.16	6.63	2.98
10	49.57	48.66	45.91	3.01	3.10	3.26
14	49.38	47.40	46.01	3.26	4.68	5.04
S/O/F -- S/O						
5	48.77	50.52	52.37	3.13	5.21	1.80
6	47.10	40.54	42.30	2.50	2.51	1.64
9	53.33	49.76	50.58	2.54	3.78	1.61
13	42.76	46.86	50.52	3.63	4.52	1.53

cont'd...

Appendix 19 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	22:4			22:5		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	1.87	2.62	2.52	4.86	3.90	6.41
7	2.02	2.10	0.96	3.86	2.78	1.76
12	2.13	1.41	3.71	5.44	2.45	8.81
SOY -- CAN						
2	1.07	0.42	0.83	2.80	1.93	3.26
3	3.17	2.46	1.64	8.53	6.49	6.68
11	0.91	1.15	1.38	3.85	3.17	3.77
15	3.17	2.10	0.78	7.21	4.54	3.04
CAN -- S/O/F						
4	2.77	2.03	1.97	6.05	6.69	5.40
8	1.53	0.00	0.65	4.02	2.20	2.71
10	2.44	1.09	1.19	5.84	5.03	6.35
14	2.19	1.21	1.35	6.55	5.22	6.04
S/O/F -- S/O						
5	3.39	2.44	2.73	7.05	7.06	5.14
6	2.10	2.02	1.71	4.09	4.92	3.45
9	3.55	2.11	2.06	6.28	5.46	3.60
13	1.10	1.75	1.27	3.78	5.79	2.22

cont'd...

Appendix 19 (cont'd).

Group / Subject	Fatty Acid (% of total) 22:6		
	Day: 7	25	43
S/O -- SOY			
1	18.86	14.50	20.05
7	14.82	16.22	12.60
12	11.25	7.10	18.13
SOY -- CAN			
2	6.93	6.44	10.38
3	18.33	19.21	16.84
11	7.06	7.95	8.40
15	11.26	7.91	6.22
CAN -- S/O/F			
4	12.21	19.49	12.02
8	22.33	15.61	16.88
10	12.77	10.83	10.26
14	9.03	9.26	8.77
S/O/F -- S/O			
5	12.98	12.80	13.59
6	20.95	21.20	22.61
9	10.89	10.08	9.09
13	14.85	16.27	9.04

Appendix 20. The Fatty Acid Composition of Plasma Cholesteryl Esters for Individual Subjects During the Study.

Group / Subject	Fatty Acid[1] (% of total) 16:0			16:1		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	11.05	11.03	11.15	3.31	2.20	0.97
7	11.06	10.17	9.33	3.08	2.85	1.98
12	11.99	10.73	9.51	4.20	2.57	1.73
SOY -- CAN						
2	13.64	10.26	10.52	2.74	1.70	1.74
3	11.11	10.05	9.06	2.24	1.00	0.92
11	11.08	9.56	9.66	1.75	1.01	1.27
15	11.61	9.97	10.69	3.39	0.92	1.60
CAN -- S/O/F						
4	12.75	10.40	10.61	2.42	1.60	1.47
8	12.01	10.16	10.44	2.62	1.53	1.84
10	12.79	10.96	10.62	3.00	2.36	2.52
14	12.33	10.14	11.06	2.34	1.13	0.96
S/O/F -- S/O						
5	12.63	9.44	10.67	2.61	0.70	0.94
6	12.26	8.75	10.21	2.16	1.26	1.28
9	11.52	10.69	10.78	4.60	2.43	3.40
13	11.94	8.96	9.66	1.33	0.57	0.68

[1] Carbon chain length : number of double bonds

cont'd...

Appendix 20 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	18:0			18:1		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	1.18	0.87	0.91	20.40	23.26	10.33
7	1.24	1.21	1.08	21.15	23.58	12.98
12	1.46	1.12	1.05	21.09	25.04	11.71
SOY -- CAN						
2	2.05	1.27	1.03	22.38	12.84	21.39
3	1.28	1.18	0.80	19.58	10.59	20.06
11	1.27	1.21	0.82	21.56	12.38	22.20
15	1.32	1.01	0.75	20.57	11.67	19.75
CAN -- S/O/F						
4	1.86	1.43	1.15	21.33	23.33	16.18
8	1.20	0.76	0.87	20.25	19.46	17.90
10	1.51	1.22	1.13	23.30	24.40	17.89
14	1.39	0.85	1.56	19.77	21.91	17.22
S/O/F -- S/O						
5	1.26	0.82	0.87	19.31	12.02	20.04
6	1.30	0.77	0.78	20.42	14.68	22.70
9	1.03	1.09	0.92	20.30	17.97	22.87
13	1.25	0.90	0.78	18.96	13.05	22.52

cont'd...

Appendix 20 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	18:2			18:3		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	51.98	50.03	64.27	0.57	0.38	1.00
7	50.46	49.16	62.39	0.41	0.28	0.89
12	51.29	50.43	66.26	0.62	0.41	0.93
SOY -- CAN						
2	48.81	62.05	52.83	0.40	0.96	1.30
3	55.95	67.58	57.93	0.44	1.05	1.64
11	56.72	68.26	57.14	0.51	1.06	1.26
15	55.24	67.23	59.00	0.39	0.78	1.15
CAN -- S/O/F						
4	52.60	53.31	60.72	0.46	1.42	2.09
8	52.01	53.55	57.35	0.47	1.16	0.99
10	53.43	53.96	59.80	0.50	1.02	1.84
14	56.60	57.31	61.51	0.39	1.35	2.49
S/O/F -- S/O						
5	54.89	66.30	58.12	0.48	2.39	0.36
6	55.56	66.51	59.29	0.45	2.80	0.35
9	52.83	57.73	51.81	0.41	1.94	0.36
13	55.88	64.16	54.82	0.38	2.37	0.27

cont'd...

Appendix 20 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	20:3			20:4		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	0.97	1.14	0.78	8.72	9.71	8.93
7	0.84	0.74	0.50	10.13	9.98	9.43
12	0.81	0.90	0.60	6.83	7.72	6.97
SOY -- CAN						
2	0.75	0.47	0.52	7.81	9.05	8.66
3	0.86	0.37	0.43	7.66	7.26	7.31
11	0.51	0.35	0.40	5.60	5.49	5.82
15	0.82	0.58	0.40	5.89	6.85	5.69
CAN -- S/O/F						
4	0.58	0.48	0.42	6.87	6.50	5.97
8	0.71	1.26	0.56	8.92	9.73	8.25
10	0.82	0.76	0.80	4.15	4.22	4.27
14	0.56	0.39	0.34	5.83	5.79	4.22
S/O/F -- S/O						
5	0.77	0.48	0.67	6.90	6.11	7.46
6	0.45	0.37	0.57	6.09	3.93	3.82
9	0.78	0.46	0.70	7.55	6.35	8.19
13	0.52	0.34	0.61	7.61	7.58	9.53

cont'd...

Appendix 20 (cont'd).

Group / Subject	Fatty Acid (% of total)					
	20:5			22:6		
Day:	7	25	43	7	25	43
S/O -- SOY						
1	0.93	0.53	0.86	0.88	0.85	0.82
7	0.73	1.00	0.57	0.90	1.03	0.85
12	1.10	0.47	0.64	0.61	0.61	0.59
SOY -- CAN						
2	0.71	0.83	1.43	0.72	0.60	0.60
3	0.58	0.45	1.29	0.29	0.47	0.56
11	0.51	0.32	0.81	0.49	0.35	0.61
15	0.48	0.52	0.65	0.30	0.47	0.32
CAN -- S/O/F						
4	0.56	1.03	0.82	0.58	0.50	0.57
8	0.77	1.32	0.87	1.04	1.09	0.93
10	0.35	0.67	0.58	0.15	0.43	0.46
14	0.56	0.88	0.48	0.23	0.26	0.15
S/O/F -- S/O						
5	0.62	1.23	0.36	0.54	0.52	0.51
6	0.39	0.35	0.44	0.92	0.57	0.57
9	0.54	0.92	0.44	0.44	0.43	0.53
13	0.86	1.14	0.27	1.28	0.92	0.85

Appendix 21. T Values Obtained from Comparison of Mean Plasma Lipid and Lipoprotein Levels for the Mixed Fat and Each of the Experimental Fat Diets.

Plasma Lipid	Diet (Day)			
	MF (7) v. S/O (25)		SOY (43) v. MF (49)	
TC	2.53	NS[1]	-1.09	NS
LDL-C	2.32	NS	0.04	NS
HDL-C	6.65	*	-0.67	NS
VLDL-C	2.17	NS	-2.94	NS
TG	-0.38	NS	-11.20	*
	MF (7) v. SOY (25)		CAN (43) v. MF (49)	
TC	4.92	*	-10.71	*
LDL-C	8.26	*	-4.43	*
HDL-C	0.79	NS	-1.73	NS
VLDL-C	2.59	NS	-1.72	NS
TG	2.64	NS	-2.82	NS
	MF (7) v. CAN (25)		S/O/F (43) v. MF (49)	
TC	8.16	*	-1.32	NS
LDL-C	4.29	*	0.32	NS
HDL-C	2.81	NS	-0.87	NS
VLDL-C	-0.08	NS	-2.29	NS
TG	-0.36	NS	-1.99	NS

cont'd...

Appendix 21 (cont'd).

	MF (7) v. S/O/F (25)		S/O (43) v. MF (49)	
TC	1.90	NS	-3.16	NS
LDL-C	2.13	NS	-3.59	*
HDL-C	1.60	NS	-1.76	NS
VLDL-C	-0.44	NS	-0.26	NS
TG	-0.13	NS	-0.74	NS

[1] When n=4, a significant difference (*) is observed if $|T| > 3.18$ at $\alpha=0.05$. Otherwise, it is not significant (NS).
When n=3, a significant difference (*) is observed if $|T| > 4.30$ at $\alpha=0.05$. Otherwise, it is not significant (NS).
n=4 for all diet sequences except S/O -> SOY.

Appendix 22. T Values Obtained from Comparison of Mean Plasma Phosphatidylcholine Levels for the Mixed Fat and Each of the Experimental Fat Diets.

Fatty Acid(s)[1]	MF v. S/O		MF v. CAN		MF v. SOY		MF v. S/O/F	
16:0	0.38	NS[2]	1.27	NS	-2.22	NS	-1.12	NS
18:0	-0.79	NS	-0.58	NS	-0.89	NS	-1.29	NS
18:1	-0.73	NS	-2.34	NS	20.32	*	13.12	*
18:2	1.12	NS	0.87	NS	-14.29	*	-1.08	NS
18:3	2.48	NS	-4.10	*	-11.60	*	-19.16	*
20:3	-0.28	NS	2.03	NS	9.69	*	2.95	NS
20:4	-1.93	NS	-0.72	NS	0.72	NS	2.96	NS
20:5	29.44	*	-3.34	*	3.18	*	-4.38	*
22:4	-1.02	NS	1.91	NS	3.95	*	0.32	NS
22:5	1.15	NS	-1.00	NS	3.08	NS	-1.15	NS
22:6	-1.43	NS	-0.60	NS	2.67	NS	0.54	NS
n-6[3]	-1.60	NS	0.02	NS	3.65	*	3.18	*
n-3[4]	-0.58	NS	-1.47	NS	2.88	NS	-0.18	NS

[1] Carbon chain length : number of double bonds
[2] When n=4, a significant difference (*) is observed if $|T| > 3.18$ at $\alpha=0.05$. Otherwise, it is not significant (NS).
When n=3, a significant difference (*) is observed if $|T| > 4.30$ at $\alpha=0.05$. Otherwise, it is not significant (NS).
n=4 for all diet sequences except S/O -> SOY.
[3] n-6 = 20:3 + 20:4 + 22:4
[4] n-3 = 20:5 + 22:5 + 22:6

Appendix 23. T Values Obtained from Comparison of Mean Plasma Phosphatidylethanolamine Levels for the Mixed Fat and Each of the Experimental Fat Diets.

Fatty Acid(s)[1]	MF v. S/O		MF v. CAN		MF v. SOY		MF v. S/O/F	
16:0	3.27	NS[2]	1.69	NS	2.10	NS	5.08	*
18:0	8.41	*	-0.48	NS	-1.33	NS	-2.08	NS
18:1	-4.42	*	-2.70	NS	2.33	NS	-0.55	NS
18:2	5.09	*	1.22	NS	-3.53	*	-3.03	NS
18:3	5.00	*	-4.11	*	-6.14	*	-20.98	*
20:3	-3.04	NS	1.35	NS	2.37	NS	1.20	NS
20:4	-0.95	NS	1.20	NS	-0.53	NS	1.72	NS
20:5	8.32	*	-4.22	*	1.68	NS	-6.89	*
22:4	-0.18	NS	12.94	*	0.26	NS	3.60	*
22:5	16.63	*	0.48	NS	0.08	NS	-3.09	NS
22:6	1.17	NS	-0.47	NS	-0.46	NS	1.09	NS
n-6[3]	-1.10	NS	1.53	NS	-0.17	NS	2.04	NS
n-3[4]	2.32	NS	-0.83	NS	-0.27	NS	0.04	NS

[1] Carbon chain length : number of double bonds
[2] When n=4, a significant difference (*) is observed if $|T| > 3.18$ at $\alpha=0.05$. Otherwise, it is not significant (NS).
When n=3, a significant difference (*) is observed if $|T| > 4.30$ at $\alpha=0.05$. Otherwise, it is not significant (NS).
n=4 for all diet sequences except S/O -> SOY.
[3] n-6 = 20:3 + 20:4 + 22:4
[4] n-3 = 20:5 + 22:5 + 22:6

Appendix 24. T Values Obtained from Comparison of Mean Plasma Alkenylacyl Ethanolamine Phosphoglyceride Levels for the Mixed Fat and Each of the Experimental Fat Diets.

Fatty Acid(s)[1]	MF v. S/O		MF v. CAN		MF v. SOY		MF v. S/O/F	
18:1	-2.62	NS[2]	-1.36	NS	2.30	NS	1.86	NS
18:2	-1.16	NS	0.04	NS	-3.56	*	-0.91	NS
18:3	1.17	NS	-3.90	*	-2.80	NS	-21.64	*
20:3	0.63	NS	12.92	*	5.38	*	4.14	*
20:4	-0.82	NS	0.42	NS	0.43	NS	0.44	NS
20:5	7.10	*	-2.19	NS	24.23	*	-2.46	NS
22:4	-0.09	NS	6.45	*	1.97	NS	0.98	NS
22:5	2.55	NS	1.56	NS	3.29	*	-0.84	NS
22:6	1.26	NS	0.10	NS	0.52	NS	-0.36	NS
n-6[3]	-0.69	NS	3.38	*	1.69	NS	0.99	NS
n-3[4]	2.35	NS	-0.14	NS	2.55	NS	-1.76	NS

[1] Carbon chain length : number of double bonds
[2] When n=4, a significant difference (*) is observed if $|T| > 3.18$ at $\alpha=0.05$. Otherwise, it is not significant (NS).
When n=3, a significant difference (*) is observed if $|T| > 4.30$ at $\alpha=0.05$. Otherwise, it is not significant (NS).
n=4 for all diet sequences except S/O -> SOY.
[3] n-6 = 20:3 + 20:4 + 22:4
[4] n-3 = 20:5 + 22:5 + 22:6

Appendix 25. T Values Obtained from Comparison of Mean Plasma Cholesteryl Esters Levels for the Mixed Fat and Each of the Experimental Fat Diets.

Fatty Acid(s)[1]	MF v. S/O		MF v. CAN		MF v. SOY		MF v. S/O/F	
16:0	1.97	NS[2]	16.00	*	3.73	*	4.31	*
18:0	2.30	NS	8.27	*	1.89	NS	2.42	NS
18:1	-6.81	*	-1.65	NS	64.57	*	5.04	*
18:2	4.33	*	-3.85	*	-30.85	*	-5.94	*
18:3	7.35	*	-7.23	*	-11.06	*	-11.56	*
20:3	-0.67	NS	-0.33	NS	4.15	*	3.97	*
20:4	-1.58	NS	-0.47	NS	-1.06	NS	2.36	NS
20:5	0.94	NS	-7.25	*	0.55	NS	-2.28	NS
22:6	-0.68	NS	-0.93	NS	-0.26	NS	1.88	NS
n-6[3]	-1.42	NS	-0.42	NS	-0.30	NS	2.97	NS
n-3[4]	0.70	NS	-7.25	*	0.15	NS	-0.56	NS

[1] Carbon chain length : number of double bonds
[2] When n=4, a significant difference (*) is observed if $|T| > 3.18$ at $\alpha=0.05$. Otherwise, it is not significant (NS).
When n=3, a significant difference (*) is observed if $|T| > 4.30$ at $\alpha=0.05$. Otherwise, it is not significant (NS).
n=4 for all diet sequences except S/O -> SOY.
[3] n-6 = 20:3 + 20:4
[4] n-3 = 20:5 + 22:6

Appendix 26. Levels of Significance for Diet Means of Fatty Acids in Plasma Phosphatidylcholine (Day 25).

Fatty[1] Acid(s)	GLM[2] PR > F	Diet Comparison					
		CAN v. S/O	CAN v. S/O/F	CAN v. SOY	S/O v. S/O/F	S/O v. SOY	S/O/F v. SOY
16:0	0.2726						
18:0	0.3740						
18:1	0.0002	0.1660	0.0003	0.0001	0.0077	0.0011	0.2446
18:2	0.0041	0.0447	0.8129	0.0169	0.0301	0.0005	0.0260
18:3	0.0001	0.0001	0.0001	0.0321	0.0001	0.0045	0.0001
20:3	0.0031	0.2773	0.0087	0.0114	0.0018	0.0023	0.8816
20:4	0.6515						
20:5	0.0083	0.0035	0.1826	0.0041	0.0362	0.7297	0.0507
22:4	0.6290						
22:5	0.0993						
22:6	0.4331						
n-6[3]	0.3197						
n-3[4]	0.1254						

[1] Carbon chain length : number of double bonds
[2] General Linear Models Procedure
[3] n-6 = 20:3 + 20:4 + 22:4
[4] n-3 = 20:5 + 22:5 + 22:6

Appendix 27. Levels of Significance for Diet Means of Fatty Acids in Plasma Phosphatidylcholine (Day 43).

Fatty[1] Acid(s)	GLM[2] PR > F	Diet Comparison					
		CAN v. S/O	CAN v. S/O/F	CAN v. SOY	S/O v. S/O/F	S/O v. SOY	S/O/F v. SOY
16:0	0.4216						
18:0	0.1045						
18:1	0.0001	0.1704	0.0001	0.0001	0.0003	0.0001	0.0040
18:2	0.1298						
18:3	0.0002	0.0004	0.1381	0.0360	0.0001	0.0500	0.0026
20:3	0.5837						
20:4	0.7512						
20:5	0.0136	0.0024	0.2367	0.0358	0.0217	0.2403	0.2434
22:4	0.9333						
22:5	0.2113						
22:6	0.8842						
n-6[3]	0.8593						
n-3[4]	0.2850						

[1] Carbon chain length : number of double bonds
[2] General Linear Models Procedure
[3] n-6 = 20:3 + 20:4 + 22:4
[4] n-3 = 20:5 + 22:5 + 22:6

Appendix 28. Levels of Significance for Diet Means of Fatty Acids in Plasma Phosphatidylethanolamine (Day 25).

Fatty[1] Acid(s)	GLM[2] PR > F	Diet Comparison					
		CAN v. S/O	CAN v. S/O/F	CAN v. SOY	S/O v. S/O/F	S/O v. SOY	S/O/F v. SOY
16:0	0.7692						
18:0	0.3310						
18:1	0.0133	0.4231	0.0433	0.0167	0.0133	0.0055	0.6030
18:2	0.0405	0.5293	0.0636	0.0467	0.0266	0.0198	0.8624
18:3	0.0001	0.0004	0.0001	0.6154	0.0001	0.0010	0.0001
20:3	0.0261	0.3425	0.0438	0.0701	0.0101	0.0158	0.7921
20:4	0.3884						
20:5	0.0003	0.0001	0.0296	0.0002	0.0048	0.3829	0.0168
22:4	0.0888						
22:5	0.0490	0.0437	0.9192	0.3351	0.0369	0.0083	0.3852
22:6	0.6178						
n-6[3]	0.2680						
n-3[4]	0.4347						

[1] Carbon chain length : number of double bonds
[2] General Linear Models Procedure
[3] n-6 = 20:3 + 20:4 + 22:4
[4] n-3 = 20:5 + 22:5 + 22:6

Appendix 29. Levels of Significance for Diet Means of Fatty Acids in Plasma Phosphatidylethanolamine (Day 43).

Fatty[1] Acid(s)	GLM[2] PR > F	Diet Comparison					
		CAN v. S/O	CAN v. S/O/F	CAN v. SOY	S/O v. S/O/F	S/O v. SOY	S/O/F v. SOY
16:0	0.8503						
18:0	0.0330	0.4568	0.0349	0.0093	0.1305	0.0334	0.3784
18:1	0.0001	0.0151	0.0001	0.0001	0.0006	0.0002	0.3334
18:2	0.4909						
18:3	0.0035	0.0027	0.4675	0.1046	0.0008	0.1015	0.0314
20:3	0.2949						
20:4	0.9259						
20:5	0.0004	0.0002	0.4136	0.0017	0.0016	0.2998	0.0066
22:4	0.0030	0.0019	0.0044	0.0009	0.6407	0.4510	0.2459
22:5	0.0783						
22:6	0.7999						
n-6[3]	0.8016						
n-3[4]	0.5005						

[1] Carbon chain length : number of double bonds
[2] General Linear Models Procedure
[3] n-6 = 20:3 + 20:4 + 22:4
[4] n-3 = 20:5 + 22:5 + 22:6

Appendix 30. Levels of Significance for Diet Means of Fatty Acids in Plasma Alkenylacyl Ethanolamine Phosphoglyceride (Day 25).

Fatty[1] Acid(s)	GLM[2] PR > F	Diet Comparison					
		CAN v. S/O	CAN v. S/O/F	CAN v. SOY	S/O v. S/O/F	S/O v. SOY	S/O/F v. SOY
18:1	0.0165	0.3867	0.0386	0.0292	0.0106	0.0081	0.8773
18:2	0.0624						
18:3	0.0304	0.0222	0.3883	0.8725	0.0050	0.0291	0.3108
20:3	0.1534						
20:4	0.8248						
20:5	0.0033	0.0022	0.3878	0.0023	0.0096	0.7547	0.0113
22:4	0.2332						
22:5	0.1641						
22:6	0.6204						
n-6[3]	0.7075						
n-3[4]	0.1564						

[1] Carbon chain length : number of double bonds
[2] General Linear Models Procedure
[3] n-6 = 20:3 + 20:4 + 22:4
[4] n-3 = 20:5 + 22:5 + 22:6

Appendix 31. Levels of Significance for Diet Means of Fatty Acids in Plasma Alkenylacyl Ethanolamine Phosphoglyceride (Day 43).

Fatty[1] Acid(s)	GLM[2] PR > F	Diet Comparison					
		CAN v. S/O	CAN v. S/O/F	CAN v. SOY	S/O v. S/O/F	S/O v. SOY	S/O/F v. SOY
18:1	0.0112	0.7533	0.0277	0.0041	0.0489	0.0070	0.2336
18:2	0.6138						
18:3	0.0002	0.0212	0.0013	0.2967	0.0001	0.1919	0.0004
20:3	0.6826						
20:4	0.8732						
20:5	0.0005	0.0001	0.1605	0.0012	0.0016	0.3887	0.0132
22:4	0.1697						
22:5	0.5634						
22:6	0.3805						
n-6[3]	0.7573						
n-3[4]	0.5924						

[1] Carbon chain length : number of double bonds
[2] General Linear Models Procedure
[3] n-6 = 20:3 + 20:4 + 22:4
[4] n-3 = 20:5 + 22:5 + 22:6

Appendix 32. Levels of Significance for Diet Means of Fatty Acids in Plasma Cholesteryl Esters (Day 25).

Fatty[1] Acid(s)	GLM[2] PR > F	Diet Comparison					
		CAN v. S/O	CAN v. S/O/F	CAN v. SOY	S/O v. S/O/F	S/O v. SOY	S/O/F v. SOY
16:0	0.0622						
18:0	0.3444						
18:1	0.0001	0.2642	0.0001	0.0001	0.0001	0.0001	0.0797
18:2	0.0001	0.0520	0.0007	0.0001	0.0001	0.0001	0.2151
18:3	0.0001	0.0003	0.0001	0.1038	0.0001	0.0040	0.0001
20:3	0.0413	0.2721	0.0845	0.1148	0.0142	0.0192	0.8578
20:4	0.1601						
20:5	0.1949						
22:6	0.3287						
n-6[3]	0.1250						
n-3[4]	0.3834						

[1] Carbon chain length : number of double bonds
[2] General Linear Models Procedure
[3] n-6 = 20:3 + 20:4
[4] n-3 = 20:5 + 22:6

Appendix 33. Levels of Significance for Diet Means of Fatty Acids in Plasma Cholesteryl Esters (Day 43).

Fatty[1] Acid(s)	GLM[2] PR > F	Diet Comparison					
		CAN v. S/O	CAN v. S/O/F	CAN v. SOY	S/O v. S/O/F	S/O v. SOY	S/O/F v. SOY
16:0	0.4436						
18:0	0.0534						
18:1	0.0001	0.1770	0.0012	0.0001	0.0001	0.0001	0.0001
18:2	0.0062	0.7031	0.1158	0.0027	0.0598	0.0015	0.0451
18:3	0.0006	0.0019	0.0620	0.1659	0.0001	0.0452	0.0059
20:3	0.1698						
20:4	0.3208						
20:5	0.0145	0.0018	0.0517	0.0701	0.0851	0.1051	0.9890
22:6	0.4806						
n-6[3]	0.2959						
n-3[4]	0.2085						

[1] Carbon chain length : number of double bonds
[2] General Linear Models Procedure
[3] n-6 = 20:3 + 20:4
[4] n-3 = 20:5 + 22:6