THE EFFECT OF DIETARY OLEIC, LINOLEIC AND LINOLENIC ACIDS ON PLASMA LIPID METABOLISM IN HEALTHY YOUNG MEN

BY

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TABLE OF CONTENTS

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 8 normolipidemic men. The study consisted 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 13% of total energy (3200 kcal/day) as protein, 53% as carbohydrate and 34% as fat. Added fat (77% of total dietary fat) from a mixture of fats was used during the pre- and post-experimental periods. Four sources of added fat, namely i)sunflower and olive (S/O), ii)canola (CAN), iii)soybean (SOY) and iv)sunflower, olive and flaxseed (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. Fasting (12-hour) blood samples were analyzed for plasma lipid and lipoprotein and for the fatty acid composition of phosphatidylcholine, phosphatidylethanolamine, alkenylacyl phosphatidylethanolamine, and cholesteryl esters (CEs). The experimental diets resulted in similar decreases in total, LDL- and VLDL-cholesterol levels, 18, 22 and 41%, respectively. Dietary fat source had no effect on HDL-cholesterol. 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 CEs.

The SOY and S/O/F diets were associated with significantly lower levels of eicosatrienoic acid and pooled n-6 polyunsaturated fatty acids (PUFA). The S/O/F diet was associated with significantly lower levels of arachidonic acid, whereas the CAN diet was associated with significantly lower levels of docosatetraenoic acid. The CAN and S/O/F diets were associated with significantly higher levels of eicosapentaenoic acid, docosapentaenoic acid and pooled n-3 PUFA. Diet, however, had no effect on docosahexaenoic acid. The results indicated that dietary OA, LA and LNA had similar blood cholesterol-lowering effects. Dietary fat source had an influence on plasma lipid fatty acid metabolism. Dietary LA/LNA ratio was important in influencing plasma n-3 PUFA metabolism, whereas the absolute levels of dietary LA and LNA were more important in influencing n-6 PUFA metabolism.

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LIST OF FIGURES

LIST OF TABLES

.....Cont'd

LIST OF TABLES (Cont'd)

LIST OF APPENDICES

.....Cont'd

LIST OF APPENDICES (Cont'd)

LIST OF ABBREVIATIONS

FULL NAME	ABBREVIATION
Arachidonic Acid	AA
Adenosine Phosphate	ADP
Alkenylacyl Phosphatidylethanolamine	PPE
Carbohydrates	CHO
Cholesteryl Ester	CE
Coronary Heart Disease	CHD
Canola Oil	CAN
Docosahexaenoic Acid	DHA
Docosapentaenoic Acid	DPA
Docosatetraenoic Acid	DTA
Eicosapentaenoic Acid	EPA
Eicosatrienoic Acid	ETA
High Density Lipoprotein-Cholesterol	HDL-C
Linoleic Acid	LA
Linolenic Acid	LNA
Low Density Lipoprotein-Cholesterol	LDL-C
Monounsaturated Fatty Acids	MUFA
Oleic Acid	OA
Phosphatidylcholine	PC
Phosphatidylethanolamine	PE
Phospholipid	PL
Prostacyclin	PGI
Palmitic Acid	PMA

.....Cont'd

LIST OF ABBREVIATIONS (Cont'd)

FULL NAME	ABBREVIATION
Pentadecaenoic Acid	15:0
Polyunsaturated to Saturated Fatty Acids	P/S
Stearic Acid	STEA
Standard Error	SE
Saturated Fatty Acids	SFA
Soybean Oil	SOY
Sunflower and Olive Oils	S/O
Sunflower, Olive and Flaxseed Oils	S/O/F
Thromboxane	TXA
Triglyceride	TG
Total Cholesterol	TC
Thin Layer Chromatography	TLC
Very Low Density Lipoprotein-Cholesterol	VLDL-C

1. LITERATURE REVIEW

1.1. INTRODUCTION

Coronary Heart Disease (CHD) is the leading cause of death in North America (HHS, 1988; Statistics Canada, 1988). The term CHD refers to all complications resulting from a two-step degenerative process in blood vessel. The first stage of this process involves the formation of an atherosclerotic plaque which acts to narrow the lumen of the blood vessel and to slow the flow of blood. In the second stage a clot forms on the surface of the pre-existing plaque. The clot continues to increase in size and eventually occludes the vessel.

Review of the scientific literature suggests that dietary fatty acids may play a role in preventing the advancement of CHD by inhibiting atherosclerosis and thrombogenesis (viz., platelet aggregation). A strong association between plasma cholesterol levels, particularly the low density lipoprotein-cholesterol (LDL-C), and CHD has been observed in animal, epidemiological and clinical studies. Unsaturated fatty acids have been found to lower plasma cholesterol levels and, probably, the risk of CHD. The opposite, however, is true for saturated fatty acids (SFA). It has been suggested that polyunsaturated fatty acids (PUFA) of the n-3 and n-6 families may influence the development of thrombi through the production of different types of prostaglandins and thromboxanes in the endothelial

wall and in the circulating platelets.

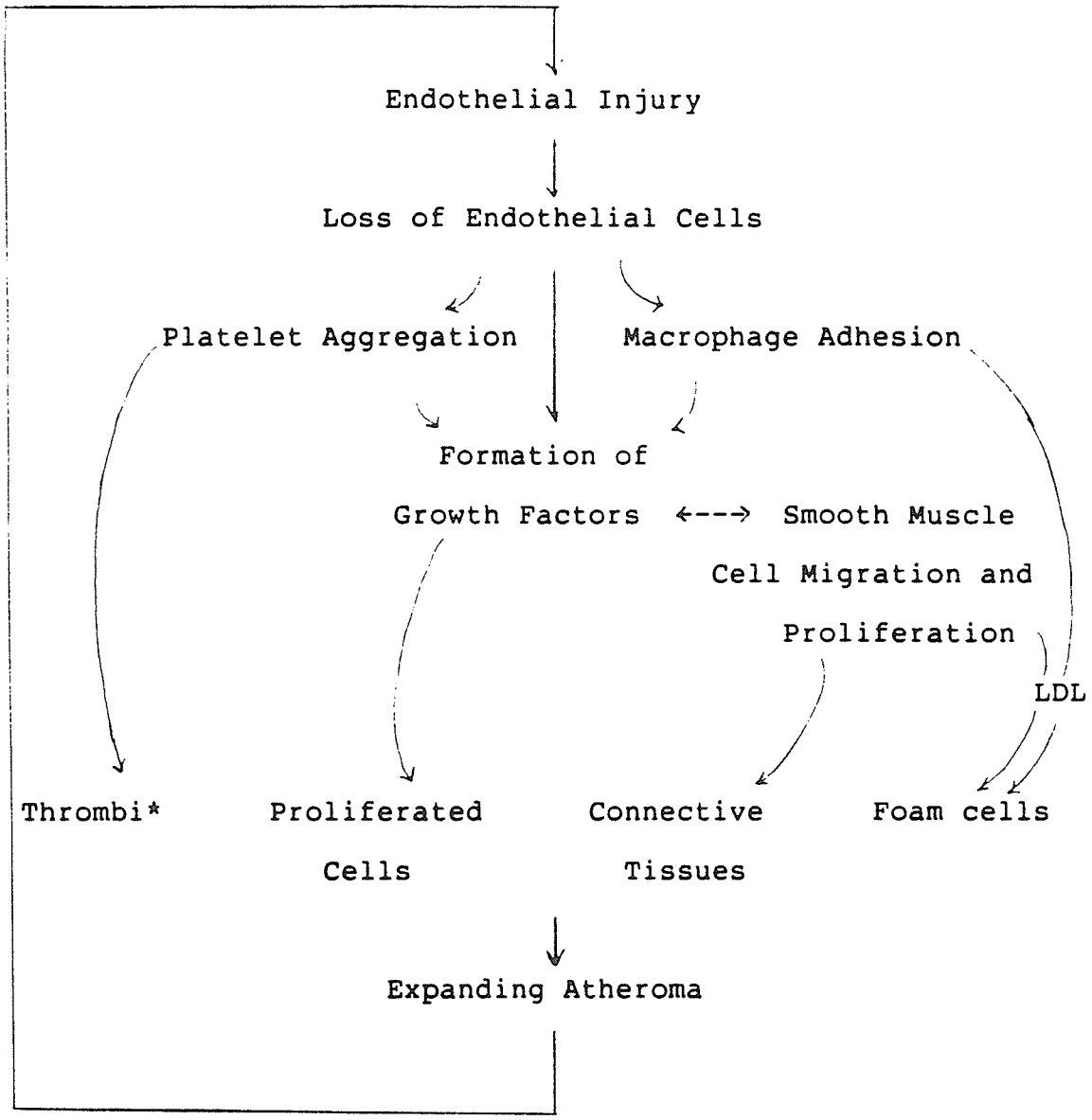
It seems possible that dietary fatty acids are capable of playing a dual role in influencing the development of CHD by exerting their effects on the two processes leading to the manifestation of the disease. Clarification of such a role should be a research priority due to the prevalence of CHD and its risk factors in most Western industrialized countries. Information obtained from continuing research work in this area would be of great help in establishing dietary goals for the general public and in the dietary management of patients suffering from the disease.

1.2. CHD - A TWO-STEP PROCESS

As mentioned previously, CHD is the result of the development of atherosclerotic plaque and thrombi in the blood vessel. A detailed description of this complicated process has been given by Leaf and Weber (1988) and Ross (1986), hence only a brief summary will be presented here (See Figure 1).

Blood vessels are lined by endothelial cells, under which are layers of smooth muscle cells. Atherosclerosis starts with damage to the endothelium. A number of factors including high levels of LDL-C, hypertension, diabetes and smoking have been implicated as the primary risk factors in the initiation of atherosclerosis. Injury to the endothelium results in the loss of endothelial cells,

Figure 1. The Development of Atherosclerosis and Thrombosis in Blood Vessel. Adapted from Leaf and Weber, 1988.



*Usually a thrombus finally occludes the vessel, terminating the process with a fatal infarction.

thereby exposing the underlying collagen to the circulating platelets. In response to activation, by collagen, platelets release thromboxane A2 (TXA2), a potent platelet aggregator and vaso-constrictor, which leads to the formation of a thrombus. Meanwhile, circulating monocytes are attracted to the damaged endothelium and are activated to become scavenger cells called macrophages. Platelet-derived growth factor is released. Similar growth factors, also, are released from the macrophages and damaged endothelial cells. The presence of these growth factors stimulate the migration of smooth muscle cells to the site of injury, where growth factors from the smooth muscle cells also are produced. Growth factors ensure the proliferation of smooth muscle cells and macrophages around the injury site. When present in high amounts, LDL-C are ingested by smooth muscle cells and macrophages. Smooth muscle cells also can form enormous amounts of connective tissue matrix (Burke and Ross, 1979). With the proliferation of cells and connective tissue and the accumulation of lipids, the lesion expands in size and gradually narrows the vessel lumen. The outpouching of the endothelium makes it more susceptible to further injury. Subsequent injury is again accompanied by platelet aggregation and cell proliferation leading to a further increase in the size of the lesion. Usually, a thrombus finally occludes the vessel, terminating the process with a fatal infarction if in a major vessel.

Platelet aggregation, therefore, is involved in both the initiation of the atherosclerotic plaque and the final arterial obstruction. Given that arterial occlusion, together with cardiac arrhythmia, has been found to be the most common event leading to lethal heart attacks (AHA, 1986), platelet aggregation may be considered as playing a more critical role than atherosclerosis in determining the outcome of the disease.

Since atherosclerosis and thrombosis are the two major steps leading to CHD, inhibition of these processes should be given serious consideration in controlling CHD.

1.3. ATHEROSCLEROSIS AND DIETARY FATTY ACIDS

1.3.1. The Relationship Between LDL and the Risk of CHD

A strong association between elevated plasma lipid levels, particularly LDL-C, and incidence of CHD has been demonstrated in animal (Ross, 1986), epidemiological (Keys, 1970; Kannell et al, 1984; HHS, 1988), and clinical (Blankenhorn et al, 1987) studies. On the other hand, decreased LDL-C level has been found to correlate with a reduction in the incidence of CHD (Lipid Research Clinics Program, 1984). Such an association could be due to the presence of a damaging effect of high levels of LDL-C on the endothelium. Jackson and Gotto (1976) suggested that elevated plasma cholesterol levels may lead to changes in the cholesterol:phospholipid ratio of the plasma membrane of

endothelial cells, which could lead to increased membrane viscosity and decreased malleability of endothelial cells. Another mechanism was proposed by Cathcart et al (1985). They found that LDL-C exposed to macrophages was oxidized and was toxic to fibroblasts in culture. If these oxidized LDL-C particles were also toxic to the endothelium in vivo, it could be an important source of endothelial injury. In addition, LDL-C may be deposited into the injured endothelium and thereby increases the size of the atherosclerotic plaque (Leaf and Weber, 1988). More research is needed before one can be sure of these suggested influences of plasma LDL-C on atherosclerosis. Nevertheless, the relationship has been reasonably well accepted and has led to concern with what factors control the level of LDL-C in circulation, which would be expected to have important implication in the prevention of CHD.

1.3.2. The Critical Role of LDL-Receptors

The primary mechanism for the clearance of LDL-C from the plasma is the LDL-receptors located on the surface of cells, particularly hepatocytes (Brown and Goldstein, 1976; Grundy, 1987). LDL-receptors bind circulating LDL-C and the resulting receptor-LDL complex is internalized into the lysosomes of the cells where the LDL-C is degraded. The majority of circulating LDL-C, about two-thirds to three-fourths, is removed by the LDL-receptor pathway; the

remainder is cleared by a nonspecific nonreceptor pathway (Grundy, 1987). The activity of LDL-receptors thus is the major factor determining the level of plasma LDL-C. When less LDL-receptors are available, the clearance of LDL-C is decreased. Since very low density lipoprotein-cholesterol (VLDL-C) remnants, the precursor of LDL-C, are also cleared by LDL-receptors, more VLDL-C remnants are converted to LDL-C when there are not enough LDL-receptors. Because of the positive relationship between plasma LDL-C and risk of CHD, it can be inferred that the LDL-receptors play a critical role in the prevention of CHD.

The significant role of LDL-receptors in atherosclerosis was first appreciated when studies showed that the absence of LDL-receptors was responsible for familial hypercholesterolemia (Brown and Goldstein, 1984). Patients with this disease have elevated LDL-C levels and develop atherosclerosis leading to various heart problems or death at an early age. Studies with animals with defective LDL-receptor genes also showed that a reduction in the number of LDL-receptors resulted in elevated plasma LDL-C levels (Brown and Goldstein, 1984). The control of the activity of LDL-receptors is, therefore, under close scrutiny by many researchers.

LDL-receptors activity is under feedback control and is inversely proportional to the cellular content of cholesterol (Brown and Goldstein, 1984; Grundy, 1987). Thus

factors that increase the cellular content of cholesterol would suppress the activity of LDL-receptors. Diet is one of these factors. For instance, dietary cholesterol has been found to suppress the activity of LDL-receptors by increasing the cellular content of cholesterol (Applebaum-Bowden et al, 1984).

1.3.3. The Effects of Dietary Fatty Acids on Plasma Lipids and Lipoproteins

Dietary fatty acids have been found capable of influencing blood lipid levels including LDL-C. Whether LDL-receptors are involved in these reactions is not clear. A number of metabolic studies were conducted by Keys et al (1957) and Hegsted et al (1965) in the 1950s and 60s to compare the effect of different dietary fatty acids on plasma total cholesterol concentrations. From the regression equations obtained, it was shown that SFA raised plasma total cholesterol (TC) level about twice as much as linoleic acid (LA) lowered it, while oleic acid (OA) was neutral in its effect on plasma cholesterol.

The cholesterol-raising effect of SFA and the cholesterol-lowering effect of LA have been confirmed in a number of studies. An epidemiological study that examined the association among diet, plasma cholesterol, other risk factors and CHD rates in several different populations within seven countries including Southern and Northern

Europe, the United States and Japan found that the dietary factor most closely correlated with a high concentration of plasma cholesterol and risk of CHD was the level of SFA intake (Keys, 1970). In laboratory animals, SFA have been shown to inhibit receptor-mediated uptake of LDL-C (Spady and Dietschy, 1988). However, it is not clear whether this is the mechanism through which SFA increase plasma LDL-C in humans. In a study conducted by Shepherd et al (1978), four normal subjects were first put on a SFA-rich diet for five weeks and then switched to a LA-rich diet. When compared with the saturated fat diet, the LA-rich diet produced significant decreases in TC, LDL-C, high density lipoprotein-cholesterol (HDL-C), VLDL-C and triglyceride (TG). Similar changes were also observed in hyperlipidemic patients after switching from a SFA-rich diet to a LA-rich diet (Vega et al, 1982).

However, the neutral role of OA on plasma cholesterol suggested by Keys et al (1957) and Hegsted et al (1965) has been questioned recently. In a study by Mattson and Grundy (1985), OA, LA and SFA were fed in the form of formula diets to hypercholesterolemic patients. Each diet was fed for four weeks. Substitution of both OA and LA for SFA resulted in similar reductions in the levels of TC and LDL-C. OA also appears to have similar cholesterol-lowering effect to complex carbohydrates (CHO) (Mensink and Katan, 1987). The effects of two diets, one rich in complex CHO and one rich

in olive oil, on serum lipids were studied in 48 healthy men and women. Results showed that serum cholesterol levels decreased to a similar extent on both diets. Similar results also were reported by Grundy (1986) and Baggio et al (1988), namely the same hypocholesterolemic effect of OA to that of a low fat, high CHO diet. A metabolic study also was conducted in our laboratory (McDonald et al, 1989) to compare the cholesterol-lowering effect of a sunflower oil-rich diet and a canola oil-rich diet. Both diets produced similar decreases in LDL-C and TC. Since sunflower oil is relatively rich in LA and canola oil is relatively rich in OA, it was assumed that these two unsaturated fatty acids had similar cholesterol-lowering effects. These results (Mattson and Grundy, 1985; Mensink and Katan, 1987; Grundy, 1986; Baggio et al, 1988; and McDonald et al, 1989) are also consistent with epidemiological data showing lower rates of CHD in the Mediterranean region, where consumption of olive oil is high (Grundy, 1987).

Contrasting results, however, were observed from a study conducted by Sirtori et al (1986) in which effects of olive oil-rich and corn oil-rich diets on plasma lipid levels were studied in 23 patients with high atherosclerotic risk. Patients were randomly assigned to one diet for eight weeks and then switched to the other in the subsequent study period. The corn oil diet was associated with significant reductions in TC and LDL-C in both periods. The olive oil

diet, by contrast, failed to produce a change in lipoprotein levels when fed first although it did maintain the reduced cholesterol levels induced by the corn oil diet.

The cholesterol-lowering effect of OA has been of interest not only because it is the major dietary fatty acid in the diet, but also because of concern for the adverse effects associated with high LA consumption. HDL-C has been described as the protective cholesterol. It is believed that HDL-C transports cholesterol away from arteries and to the liver for recycling or disposal (Brown et al, 1981). High HDL-C levels are, therefore, desirable in reducing risk for CHD. It has been shown that dietary LA lowers HDL-C, especially when it is consumed in high amounts (Shepherd et al, 1978; Shepherd et al, 1980; Grundy, 1986). Other disadvantages associated with high LA intake include the likelihood of enhancing tumor development and suppressing immune response (Grundy, 1987). LA has also been found to increase the excretion of fecal bile acid (Connor et al, 1969), and thus increases the risk of developing gall stones.

Due to the increasing interest in n-3 PUFA on the prevention of thrombosis, researchers have also looked at the cholesterol-lowering effect of these fatty acids. Eicosapentaenoic acid (EPA), a longer chain n-3 PUFA, has been found to have a consistent TG-lowering effect by inhibiting the formation of VLDL-C and its apoproteins

(Herold and Kinsella, 1986). Since VLDL-C remnants are precursors for LDL-C, decreased VLDL-C production would be expected to give rise to lowered plasma cholesterol levels. However, the effect of EPA on plasma cholesterol is not consistent and no firm conclusion can be drawn (Herold and Kinsella, 1986).

Keys et al (1965) and Lasserre et al (1985) found that a rapeseed oil-rich diet had a similar cholesterol-lowering effect as LA-rich or low-fat diets. Contrasting results, however, were reported by other investigators on the effect of α-linolenic acid (LNA), the parent compound of the n-3 PUFA series, on plasma lipids. No changes in plasma TC, LDL-C, HDL-C and TG were observed in a study in which subjects were given 20 ml linseed oil, a LNA-rich oil, per day as a supplement for two weeks (Sanders and Roshanai, 1983). Similar results were obtained from another study where subjects were provided with 30 ml linseed oil supplement per day for four weeks (Mest et al, 1983). In a long term dietary study, Renaud et al (1986) found increased LNA consumption was associated with only moderate decreases in plasma TC and TG, 9.5% and 3%, respectively.

The mechanisms by which dietary fatty acids influence plasma cholesterol levels have not been elucidated. It is believed that SFA increase plasma LDL-C by suppressing the activity of LDL-receptors (Spady and Dietschy, 1988). Grundy (1986) suggested that the cholesterol-lowering effect

of unsaturated fatty acids is the result of their replacement of LDL-receptor-suppressive SFA in the diet and consequently in the plasma. A multicenter study (International Collaborative Study Group, 1986) reported a positive correlation between dietary intake of monounsaturated fatty acids (MUFA) and the LDL-C catabolic rate in monocytes in vitro, suggesting that MUFA may exert their hypocholesterolemic effect by enhancing the LDL-receptor activity. Nestel (1987), on the other hand, states that LA may lower plasma cholesterol by a number of other mechanisms in addition to replacing SFA or enhancing LDL-receptor activity. These mechanisms include reduced formation of VLDL apoprotein B, thus reduces availability of precursor for LDL-C synthesis; alterations in the composition of LDL-C, thereby facilitating its uptake by LDL-receptors; and increased excretion of cholesterol in the form of bile.

1.4. THROMBOSIS AND DIETARY FATTY ACIDS

1.4.1. Experience of the Greenland Eskimos

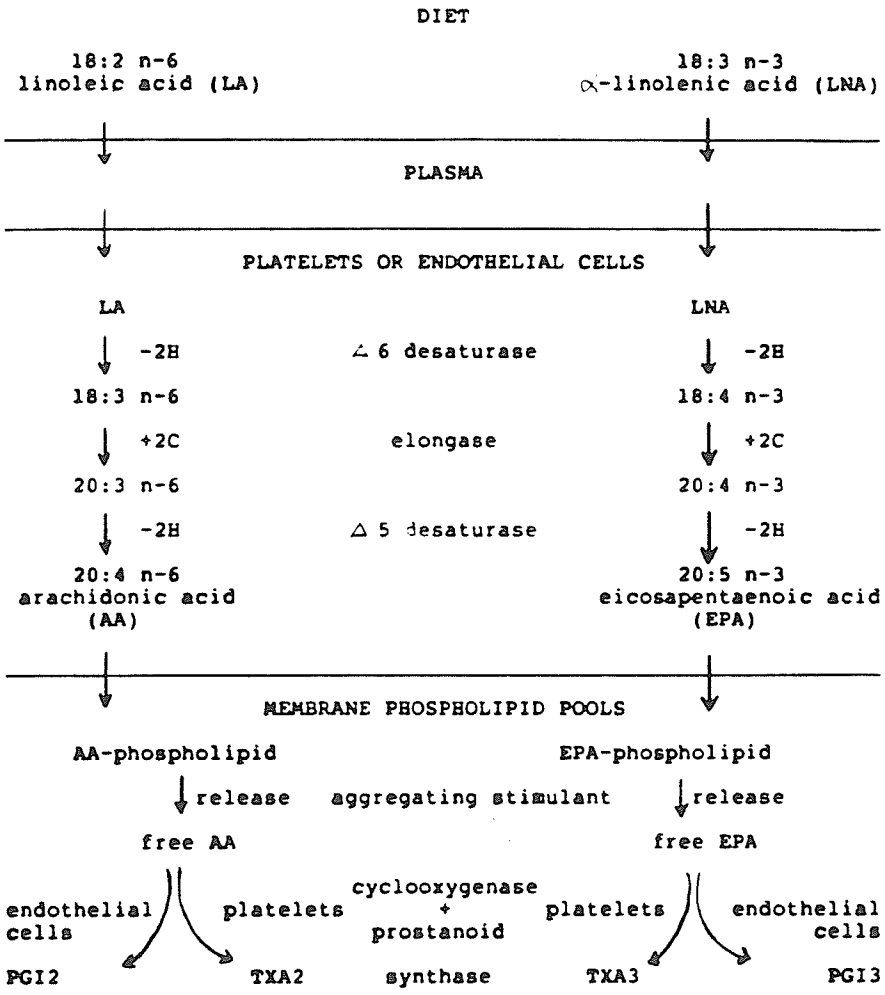
Two major families of PUFA, n-6 and n-3, have been found to be intimately involved with the production of prostanoids, hormone-like metabolites that have diverse biological functions, including the regulation of vasodilation and platelet aggregation, in the body.

Early in the 1970s, studies by Bang and Dyerberg observed that Greenland Eskimos had higher levels of antithrombogenic prostanoids in their circulation, decreased platelet aggregability, prolonged bleeding time and much lower incidences of CHD when compared with Danes and with Eskimos living in Denmark (Dyerberg, 1986). In terms of diet, the most obvious difference between these two populations was that Greenlanders consume higher amounts of seafood rich in EPA. Although other environmental factors, not just diet, may be responsible for the unusually low incidences of CHD in Greenlanders, the effect of dietary fatty acids on prostanoid metabolism and platelet function has been studied extensively since then. Mechanisms of how PUFA of the n-3 and n-6 families may interact to influence prostanoid production and platelet aggregability in the body have been unfolding in the literature (Kinsella, 1986, Smith and Borgeat, 1985).

1.4.2. The Proposed Mechanisms (Figure 2)

LA and LNA are the parent compounds of the n-6 and n-3 families of unsaturated fatty acids. All fatty acids in these families, regardless of chain length and degree of unsaturation, have the last double bond six and three carbons, respectively, from the methyl end of the fatty acid chain. The human body cannot insert double bonds more than nine carbons from the carboxyl end of the fatty acid chain.

Figure 2. Prostanoid Synthesis from Dietary Polyunsaturated Fatty Acids



Accordingly, LA and LNA are essential fatty acids, in that they must be provided through the diet. LA is the major PUFA present in the diet, whereas the conventional Western diet does not contain LNA to a great extent.

LA and LNA are ingested into the body together with other fatty acids mostly in the form of TG. Once ingested, fatty acids attached to TG, phospholipids (PLs) and cholesteryl esters (CEs) are incorporated into chylomicrons, and released into the lymphatic duct and eventually into the circulation. The majority of these dietary fatty acids are picked up by the adipose tissues in which they are stored. Eventually they are oxidized for energy purpose. Only a very small amount of the ingested fatty acids are ultimately incorporated into the PL pools of tissue membranes, including those of the endothelium and platelets (Kinsella, 1988).

Once in the endothelial cells and platelets, LA and LNA can be desaturated and elongated (Mead et al, 1986), although desaturation and elongation of these fatty acids also may occur in the liver, prior to their incorporation into tissue lipids. Both fatty acids require the same enzyme systems for desaturation and elongation. In fact, the n-3 and n-6 families compete for the enzyme pathway in such a manner that n-3 PUFA suppress the bioconversion of n-6 PUFA, while the suppressive effect of n-6 PUFA on n-3 PUFA is less severe (Ziboh and Chapkin, 1988). As shown in

Figure 2, LA and LNA will first be desaturated to 6-9-12-octadecatrienoic acid and 6-9-12-15-octadecatetraenoic acid, respectively by Δ6 desaturase. In the next step, the desaturated fatty acids will be elongated to 8-11-14-eicosatrienoic acid and 8-11-14-17 eicosatetraenoic acid, respectively by elongase. The elongated fatty acids are then desaturated to 5-8-11-14-eicosatetraenoic acid, or arachidonic acid (AA), and 5-8-11-14-17-eicosapentaenoic acid (EPA), respectively by Δ5 desaturase. The three 20-carbon PUFA derived from LA and LNA, 8-11-14-eicosatrienoic (n-6) acid, AA and EPA, are the precursors for prostanoids of the 1, 2, and 3 series, respectively.

As mentioned previously, EPA can also be supplied directly from the diet in the form of fish or fish oils. EPA in cells can be further elongated and desaturated to docosahexaenoic acid (DHA), another long chain n-3 PUFA found in high amounts in the diet of the Greenland Eskimos. The role of DHA in platelet aggregation is not as pronounced as that of EPA (Powell and Funk, 1987). However, DHA is the major PUFA found in human brain and retina, and it may play an important role in maintaining normal retinal and brain function (Tinoco et al, 1979, Sanders and Rana, 1987).

Under normal conditions, the AA and EPA derived from LA and LNA are located on the 2-acyl position of PLs of cell membranes (Leaf and Weber, 1988). They are released from the membrane PL pools in the presence of activating agents

generally referred to as aggregating stimulants. The most powerful aggregating stimulants known include arachidonic acid, thrombin, collagen, epinephrine, and adenosine phosphate (ADP) (Mead et al, 1986). In response to these stimuli, phospholipase A2, found in cell membranes, splits off the 20-carbon fatty acids from the PLs. Once released, the fatty acids are subjected to the activation of cyclooxygenase and prostanoid synthase, present in the cell membranes, leading to the formation of prostanoids.

Different prostanoids are formed depending on two conditions, namely the specific precursor or fatty acid from which the prostanoid is derived, and the specific tissue type involved. AA is the precursor of prostanoids of the 2-series, and EPA is the precursor of the prostanoids of the 3-series. In the endothelial cells, prostacyclins (PGI) are the primary products whereas TXA are formed by the platelets. TXA are vasoconstrictors and platelet aggregators; TXA2, synthesized from AA, is a potent vasoconstricting and platelet-aggregating agent, whereas TXA3, formed from EPA, is a weak vasoconstricting and aggregating agent (Leaf and Weber, 1988). PGI of both the 2 and 3 series have similar vasodilating and antiaggregating effects (Leaf and Weber, 1988).

Under normal conditions, vascular endothelium keeps platelets from adhering and initiating thrombus formation by producing an optimum amount of PGI to counterbalance the

production of proaggregatory vasoconstrictive TXA by platelets, especially that of TXA2 (Mead et al, 1986). The balance between PGI and TXA is the key in preventing the development of thrombosis. This balance seems to be subjected to a number of influences, one of them being diet.

As mentioned, PUFA of the n-3 and n-6 families share the same enzyme systems for desaturation, elongation and formation of prostanoids. The relative amounts of these two families of fatty acids present at one time, therefore, may affect prostanoid metabolism. It is proposed that high dietary intakes of n-3 PUFA will result in the production of more EPA-derived prostanoids, PGI3 and TXA3, and less AA-derived prostanoids, PGI2 and TXA2. The net result is a shift in the system towards a less thrombogenic state. A number of mechanisms have been suggested to be involved in bringing about such a change.

(1) The key rate limiting step in the conversion of LA to AA and LNA to EPA involves the Δ 6 desaturase (Brenner, 1981). This enzyme has been found to have a preference for LNA which could competitively inhibit the conversion of LA to AA, while producing increased amounts of EPA.

(2) EPA and DHA, whether converted from LNA or supplied directly from the diet, may inhibit AA formation from LA thereby reducing substrates for TXA2 synthesis

(3) EPA and DHA may compete with AA for the 2-acyl position of membrane PLs, such that plasma and cellular AA

levels are reduced and so are the levels of AA derived prostanoids

(4) EPA may compete with AA for the cyclooxygenase and thus inhibit the production of TXA2 (Leaf and Weber, 1988). It has been found in animal studies that EPA has a relatively high affinity for cyclooxygenase, but once bound it is not converted to products as effectively as AA (Powell and Funk, 1987).

1.4.3. EPA

1.4.3.1. The antithrombogenic role of EPA

Studies by Bang and Dyerberg on Greenland Eskimos found that a high intake of EPA and DHA was associated with high plasma and platelet EPA levels (Dyerberg et al, 1975, Dyerberg and Bang, 1979), increased urinary metabolites of PGI2 and PGI3, decreased production of TXB2 and TXB3 (Dyerberg et al, unpublished data), stable metabolites of TXA2 and TXA3, respectively, reduced platelet aggregability and prolonged bleeding time (Dyerberg and Bang, 1979).

Numerous studies on the effect of fish, fish oil or fish oil concentrate consumption (Herold and Kinsella, 1986) have found that high intakes of EPA can: significantly increase plasma and platelet EPA and DHA; decrease plasma LA, but not AA; and decrease platelet LA and AA contents. In terms of prostanoid metabolism, increased formation of TXB3 was observed along with a reduced formation of TXB2.

In vitro production of PGI3 from EPA of human smooth muscle cells also has been demonstrated, whereas no changes in PGI2 formation were observed. Reductions in ADP- and collagen-induced platelet aggregation and prolonged bleeding time also have been observed. These results support the suggestion that high EPA intakes suppress n-6 PUFA metabolism and lead to a decrease in thrombogenic tendency.

1.4.3.2. Adverse effects of high EPA consumption

Although the presence of high amount of EPA in the body has recently been suggested as necessary for the amelioration of thrombosis, excess consumption of the fatty acid could induce harmful effects in the body as well. High EPA consumption, usually associated with high intakes of fish oil and fish oil concentrate, may lead to adverse effects, such as, prolonged bleeding time, increased in vivo peroxidation, reduced immune responses and vitamin A and D toxicity (Leaf and Weber, 1988). Unfortunately, the definitions of optimum and excess amounts of EPA do not exist at the present time.

1.4.4. LNA

1.4.4.1. The antithrombogenic role of LNA

Although the antithrombogenic role of EPA has been studied extensively, the parent compound of the n-3 PUFA, LNA, has not generated as much attention as it may deserve.

As mentioned earlier, LNA may competitively inhibit the conversion of LA to AA and limit the availability of substrate for prothrombogenic prostanoid synthesis. In addition, LNA may act as a precursor of EPA, and thereby exert the antithrombogenic effects of EPA. Evidence obtained from animal and metabolic studies on LNA metabolism does suggest the presence of an effect of LNA on prostanoid precursor availability and platelet function.

1.4.4.2. Animal studies on LNA metabolism

Diets containing ratios of LNA:LA of 1:32, 1:7, 1:1, 3.5:1 in the form of corn oil, soybean oil, soybean/linseed oil mix and linseed oil were fed to rats for two months (Marshall and Johnston, 1982). Fatty acid analysis of liver and spleen phosphatidylethanolamine (PE) found that as the level of dietary LNA increased the level of AA decreased and EPA increased. A decrease in the synthesis of prostanoids of the 2-series in the liver also was observed. In another study (Ishinaga et al, 1983), rats were fed diets containing linseed oil, safflower oil or cocoa butter for 27 weeks. When compared with other diets, the linseed oil diet decreased the AA content and increased the EPA content in platelet PLs. The collagen-induced aggregation of washed platelets was significantly lower only in rats fed linseed oil.

1.4.4.3. Short term metabolic studies on LNA metabolism

In a study by Mest et al (1983) ten healthy volunteers were administered a 30 ml supplement of linseed oil for four weeks. This regimen led to significant increases in LNA and EPA, and significant decreases in LA and AA levels in the plasma PLs. Similar changes were obtained by Budowski et al (1984). In this study eight subjects were provided with 60 ml linseed oil daily for six weeks. This protocol led to significant increases in plasma LNA and EPA contents, whereas LA and AA levels were lower following the supplementation. According to Mest et al (1983), changes in plasma PL fatty acid composition did not lead to any changes in the in vitro TXA2 formation by platelets. Platelet prostanoid production was not measured by Budowski et al (1984), but platelet-function tests on two of the eight subjects revealed a striking decrease in platelet aggregability.

Sanders and Roshanai (1983) compared the influence of LNA and EPA on the fatty acid composition of platelet PLs in man. Five healthy males were given 20 ml linseed oil supplement per day for two weeks, and then given 20 ml MaxEPA, an EPA-rich fish oil concentrate, for two weeks after a break of six weeks. The LNA and EPA contents of platelet PLs increased significantly following the linseed oil supplementation, but the increase in EPA was less than one-third of that induced by direct EPA supplementation.

The level of AA in the platelet PLs was not affected by the feeding of LNA but was decreased by the MaxEPA supplement. Results suggested that prostanoid precursor availability could be influenced by dietary LNA, yet the changes were small compared to those obtained by direct EPA consumption.

Adam et al (1986) investigated the effect of dietary LNA on the fatty acid composition of plasma and platelet PLs. Isocaloric formula diets containing a constant amount of LA but different amounts of LNA were fed to two groups of six healthy females for two weeks each. Plasma LNA and EPA levels increased significantly as dietary LNA increased. However, no changes in plasma AA were observed. A significant increase in platelet LNA content was observed when the LA/LNA ratio of the diet was decreased to 0.25, yet only small and insignificant increase in platelet EPA were seen. No changes in platelet PL AA level were observed. Urinary prostanoids also were measured and were found to decrease with increased LNA intake.

The experimental periods associated with the studies (Sanders and Roshanai, 1983; Adam et al, 1986), however, were of short duration which may have minimized the effect of LNA on plasma and platelet fatty acid levels.

1.4.4.4. Long term dietary study with LNA

Renaud et al (1986) found, in a large group of European farmers, that an increase in dietary intake of LNA from

0.37% to 1.00% of total energy for a year was associated with significant but small increases in plasma LNA and EPA and in platelet EPA, and significant but small decreases in plasma and platelet AA. These changes, in turn, led to dramatic decreases in platelet aggregation to thrombin, ADP and collagen, and in platelet clotting activity. Results suggested that an increase in dietary LNA could lead to appreciable changes in platelet behaviour, in spite of only minor changes in plasma and platelet lipid patterns.

1.4.5. The Role of LA in the Development of Thrombosis

Although LNA can be desaturated and elongated to EPA in the body, the conversion is not of high efficiency (Sanders and Roshanai, 1983). Since LA and LNA compete for the desaturase and elongase enzymes, the large amounts of LA present in conventional diets may interfere with the formation of EPA from LNA (Leaf and Weber, 1988). At reduced intakes of LA, or a lower LA/LNA ratio, LNA may become a more effective source of EPA and thus becomes more useful in shifting prostanoid metabolism towards a less thrombogenic state. A study by Sanders and Younger (1981) on vegetarians and omnivores indicated that feeding linseed oil supplements to the subjects decreased the LA/LNA ratio of their diets to different extent. This led to significant but different increases in the EPA content in plasma PC; higher EPA levels were found with the omnivores, whose

dietary LA/LNA ratio decreased to a lower level following the supplementation. The ratio effect also was demonstrated by Adam et al (1986). However, it is less clear whether the changes in plasma and tissue fatty acid composition found in the study were due to decreases in the dietary LA/LNA ratio or to increases in LNA intake.

1.5. A SUMMARY OF THE RATIONALES BEHIND THE PRESENT STUDY

In light of the prevalence of CHD in most Western industrialized countries, the effect of dietary fatty acids on the prevention of atherosclerosis and thrombogenesis warrants continued research effort. Studies on the effect of dietary fatty acids on plasma lipid and lipoprotein levels and on platelet function suggest that dietary fatty acids can influence both processes and thus, in turn, the development of CHD. Generally, SFA have been found to be associated with elevated plasma cholesterol levels, especially the TC and cholesterol in the LDL fraction. Hence high intakes of SFA increase the risk of CHD. Dietary OA and LA, on the other hand, are regarded as being hypocholesterolemic. The effect of LNA on plasma cholesterol levels is not as clear.

Both LA and LNA may be capable of influencing thrombogenesis. Since LA and LNA are desaturated and elongated to AA and EPA, the precursors of proaggregatory and vasoconstricitve (TXA2 mainly) and antiaggregatory and

vaosdilative (TXA3 and PGI3) prostanoids, respectively, by the same enzyme systems, increasing the amount of LNA may help in shifting the homeostasis towards a less thrombogenic state.

Most of the present research work on n-3 PUFA and thrombosis has been focusing on EPA, mainly because it is the direct precursor of antithrombogenic prostanoids and a powerful inhibitor of n-6 PUFA metabolism (Sanders and Roshanai, 1983) in the body. However, due to the imperfection of the conversion, the antithrombogenic effect of LNA is not as profound as EPA, on an equivalent weight/molar basis. Yet research into the antithrombogenic role of LNA should be encouraged due to the possible adverse effects associated with high EPA consumption. In addition, LNA, which is present in relatively high amounts in certain types of vegetable oils, namely canola and soybean, represents an accessible, feasible and wholesome alternative to fish, fish oil and fish oil concentrate consumption.

Because of the competitive relationship between LA and LNA, the relative and absolute amounts of these fatty acids present in the diet would be expected to have an effect on their metabolism in the body. The importance of the LA/LNA ratio in the diet has been studied by several investigators (Marshall and Johnston, 1982, Adam et al, 1986, Sanders and Younger, 1981). It is possible that the absolute contents of LA and LNA in the diet are of importance as well. For

instance, diets with similar LA/LNA ratios but different absolute contents of LA and LNA may exert different effects on the in vivo conversion of LNA to EPA.

The effect of LNA and its ratio to LA on the fatty acid composition of plasma PLs and CEs has been investigated in the present study. A direct relationship exists between the fatty acid composition of the CEs and that of the PLs, particularly the PC; since it has been shown that the LCAT reaction in human plasma catalyzes the transfer of PUFA from the 2-acyl position of PC to free cholesterol leading to the formation of CEs (Mead et al, 1986). No direct evidence exists to show that prostanoid precursor fatty acids in plasma PLs or CEs are used for prostanoid synthesis. However, it has been shown in rabbits that lipoproteins and platelets may exchange phospholipids (Joist et al, 1976). Also, LDL-C particles are taken up by receptors in cells within which PLs and CEs attached to the particles are susceptible to various lipases, whereby prostanoid precursor fatty acids would thus be released (Brown and Goldstein, 1976; Laustiola and Salo, 1986). Based on these findings one can expect that an association exists between plasma PL and CE fatty acid composition and prostanoid production.

The fatty acid composition of plasma phosphatidylcholine (PC), diacyl phosphatidylethanolamine (PE) and alkenyacyl phosphatidylethanolamine (PPE) in particular was studied. It has been shown that the diacyl

PC, diacyl PE and PPE are the major reservoirs for EPA, AA, DHA and docosapentaenoic acid (DPA) containing PLs in platelets (Mahadevappa and Holub, 1987; Holub et al, 1988). A similar distribution pattern of these fatty acids in plasma PLs is expected. Except for AA, the other long chain PUFA do not exist in large amounts in plasma PL pools, the identification of the major storage sites for these fatty acids in the plasma aids in the study of the metabolism of these fatty acids.

2. OBJECTIVES

The direct objective of the present study was to investigate the effect of diets with different amounts of SFA, OA, LA and LNA on plasma lipid and lipoprotein patterns, and the fatty acid composition of plasma PLs (PC, PE, PPE) and CEs. Based on the above observations, it should be possible to draw a number of implications regarding

-- the effect of dietary SFA, OA, LA and LNA on plasma lipid and lipoprotein patterns,

-- the effect of dietary LA and LNA and their ratio on the fatty acid composition of plasma PLs and CEs.

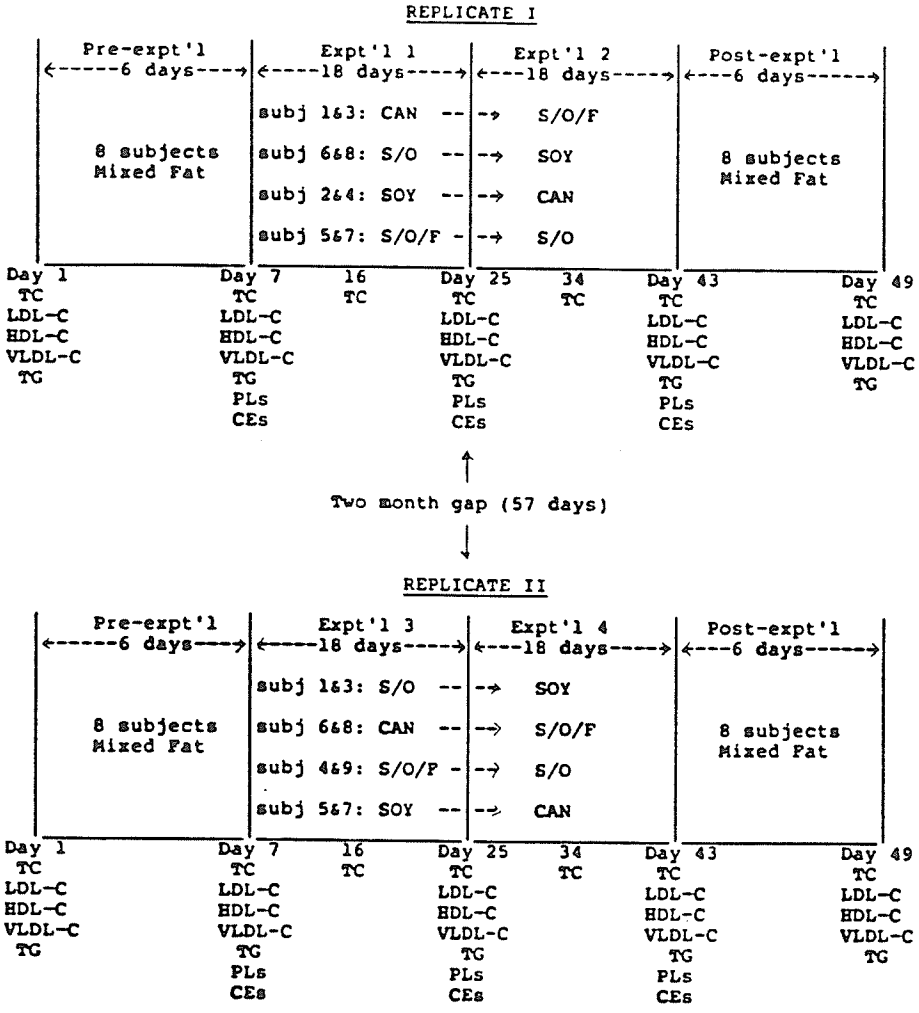
3. MATERIALS AND METHODS

3.1. EXPERIMENTAL DESIGN

The study consisted 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. There was a two-month gap between replicates. The experimental design of the study is shown in Figure 3. The eight subjects participated in the study were first divided into two groups, high and low TC, on the basis of their TC levels on Day 1 of replicate I. All subjects were given the mixed fat diet in 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 flaxseed diet (S/O/F). As a result, one subject from the group with higher TC level and one from the group with lower TC level were assigned to each diet. In the subsequent experimental periods of replicate I and II, each pair of subjects was switched to a different diet, according to the following predetermined order: S/O to SOY, SOY to CAN, CAN to S/O/F, S/O/F to S/O.

12-hour fasting blood samples were obtained from the subjects on Days 1, 7, 16, 25, 34, 43, and 49 of each replicate.

Figure 3. Experimental Design



3.2 SUBJECTS

Eight male volunteers were recruited for the study. They were students at the University of Manitoba and were selected from a group of volunteers who responded to notices posted on campus. The purpose, nature and the restrictions associated with the study were communicated to the respondents. Those that were interested in and willing to fulfill all the conditions associated with the study were invited to become subjects. The subjects also were required to pass a physical examination including normal fasting blood lipid levels prior to inclusion on the study. Subject 8 started on Day 2 of the study. Subject 2 left after the completion of replicate I, so subject 9 was recruited for replicate II. Subject 8 and 9 did not undergo a physical examination prior to the start of the study, but their plasma cholesterol levels were normal. The subjects ranged in age from 20 to 34. Physical data for the subjects 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.

The subjects came to the metabolic unit located in the Human Ecology Building on the University of Manitoba campus for their meals throughout the study. All food and beverage, including clear tea, black coffee, artificial

Table 1. Physical Data for the Subjects

Subject	Height (cm)	Initial Weight (Kg)	BMI (Kg/m2)	Screening Plasma Cholesterol[1] (mmol/L)
1	177.8	84.8	26.8	4.35
2[2]	175.3	65.8	21.4	4.09
3	162.6	65.0	24.6	4.58
4	179.1	74.8	23.3	4.87
5	167.6	54.4	19.4	4.07
6	177.8	81.2	25.7	4.24
7	180.3	63.1	19.4	4.54
8	182.9	76.2	22.8	3.80
9[2]	177.8	73.0	23.1	5.86

1 Measured two weeks prior to the start of the study.
2 Subject 2 participated in the first replicate of the study, subject 9 participated in the second replicate.

sweeteners and diet drinks, were provided throughout the study. Consumption of alcohol was prohibited. Subjects were instructed not to use aspirin and were asked to consult with the project directors before taking any medication. The subjects weighed themselves daily, at the same time each day. Other than these restrictions, the subjects engaged in their normal activities and resided in their own homes throughout the study.

3.3. DIET

A two-day cyclic menu of conventional foods was used in the study. An example of the menu is shown in Table 2. The diet was designed to provide approximately 3200 kcal per day, of which 53% was contributed by CHO, 13% by protein and 34% by fat (Table 3).

Five different diets, which were identical in all aspects except sources of added fat, were used in the study. Approximately 77% of the total fat (26% of total energy) was from added sources. 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 flaxseed oils (S/O/F diet). The composition of the added fat in each

Table 2. Two-Day Cyclic Menu[1]

	Food Items (Amounts)	
	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 Cocktail[3] Skim Milk (250 ml)
Dinner	Hamburger Patty[5] (62 g cooked) Mashed Potatoes[2,3] Carrots[2,3] Canned Pears (120 g) Skim Milk (250 ml)	Chicken and Vegetables[2,3,4] Noodles[2,3] Canned Peaches (120 g) Skim Milk (250 ml)
Snacks	Raw Apple (1 medium) Cookies (3)[2,3] Skim Milk (125 ml)	Raw Apple (1 medium) Cookies (3)[2,3] Skim Milk (125 ml)
Bread	9 slices	8 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 sweeteners 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]

Nutrients	Weight in gram	% of total energy[2]
Carbohydrate	425 g	53%
Protein	100 g	13%
Fat	119 g	34%

1 Means of twenty duplicate diet samples.
2 Total energy intake = 3170 Cal.

diet is shown in Table 4. The distribution of the added fat among the various foods in the diets is outlined in Table 5.

The saturated fatty acids, oleic acid, linoleic acid and linolenic acid contents 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 to total energy intake are shown in Appendix 2, 3 and 4, respectively. The experimental diets contained about half the level of saturated fatty acids present in the mixed fat diet. The S/O and the CAN diets had similar amounts of OA and LA, but the latter contained about eight times more LNA. The SOY diet contained about the same amount of LNA as the CAN diet, but more than twice the level of LA. As a consequence, the OA content of the SOY diet was about half that of the CAN diet. The S/O/F diet had about the same LA/LNA ratio as the CAN diet, 2.7:1 and 3.0:1, respectively, but the LA and LNA levels were approximately twice those of the CAN diet. The level of dietary cholesterol was approximately 100 mg higher in the mixed fat diet as a result of the presence of more animal fat sources in this diet.

Food intakes of the subjects were modified whenever persistent weight changes occurred. When changes were necessary, special attention was taken to maintain constant the fatty acid and macronutrient proportions of the diet.

Table 4. Sources of Added Dietary Fat[1]

Diets	Fat Sources
Mixed Fat[2]	- 11% corn oil, 22% lard, 22% tallow, 22% shortening, and 22% butter
S/O[3]	- 20% sunflower and 80% olive oils
CAN[4]	- 100% canola oil
SOY[5]	- 100% soybean oil
S/O/F[6]	- 47% sunflower, 20% olive, and 33% flaxseed oils

1 Accounted for approximately 77% of total dietary fat. The remaining 23% 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.
Beatrice Modern Butter, Modern Dairies Ltd., St Claude, 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 Flaxseed oil, Omega Nutrition Inc., Vancouver, BC.

Table 5. The Distribution of Added Fat in Different Diets.

Food Items	Diets				
	Mixed[1]	S/O	CAN	SOY	S/O/F[2]
	Amount per Day (g)				
Menu I[3]					
Granola	12	12	12	12	12
Salad Dressing	5	5	5	5	
Rice	10	10	10	10	9
Instant mashed Potatoes	26	26	26	26	27
Carrots	6	6	6	6	5
Cookies	18	18	18	18	18
Spread[4]	22	22	22	22	26
Menu II[3]					
Granola	12	12	12	12	12
Salad Dressing	5	5	5	5	
Chili	21	21	21	21	26
Chicken & Vegetables	16	16	16	16	10
Noodles	5	5	5	5	5
Cookies	18	18	18	18	18
Spread[4]	22	22	22	22	26

1 5 g/d of corn oil was consumed as salad oil and 28 g/d of butter (22 g/d of butter fat) was consumed as spread. Lard, tallow, shortening, 22 g/d each, and 6 g/d of corn oil were blended to an uniform consistency and used in baking and preparing other food items shown here.

(Cont'd)

Table 5 (Cont'd).

2 6 g/d of flaxseed oil was added to either instant mashed potatoes or chili immediately prior to serving, and the remaining 26 g/d was made into a spread with butter. 30 g/d of sunflower oil was used in baked goods. An additional 16 g/d of sunflower oil was mixed with 18 g/d of olive oil and used in preparing other 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/d of butter (11 g/d of butter fat) was used in the S/O, CAN and SOY diets, whereas 16 g/d of butter (13 g/d of butter fat) was used in the S/O/F diet.

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

	Diets				
Fatty Acids	Mixed Fat	S/O	CAN	SOY	S/O/F
	(Percent of total fat)				
SFA	41.0	19.9	16.1	21.8	19.6
OA	38.5	56.0	54.2	24.8	29.6
LA	12.0	21.9	19.5	44.9	36.0
LNA	1.0	0.8	6.6	6.5	13.4
P/S Ratio[2]	0.3	1.2	1.6	2.4	2.5
LA/LNA Ratio	12.0	27.4	3.0	6.9	2.7

1 Means of four duplicate diet samples.
2 P/S ratio = LA+LNA/SFA.

3.4. HANDLING, PREPARATION AND STORAGE OF FOOD ITEMS

All food, except fresh produce, milk and bread which were purchased from a single nearby supplier on a bi-weekly basis, were purchased as single lots. Fresh produce and milk were stored at 7 ^{o}C in a conventional refrigerator while the bread was stored at -10 ^{o}C until required for use. Canned goods, dry staples and frozen juice and vegetables were purchased from local suppliers and were stored under conditions appropriate for each item. Shortening, lard, beef tallow, corn oil and butter were purchased from nearby stores and also were stored in the walk-in refrigerator. The oils used in the experimental diets, except for the flaxseed oil which was stored at -10 ^{o}C, were stored in sealed containers at 7 ^{o}C in a walk-in refrigerator.

Food preparation started approximately two months prior to the start of the study. Granola and cookies were baked and packaged. The ground beef used for chili and the chicken breast used for casseroles and sandwiches were cooked and portioned. Hamburger patties were portioned raw. All these items were stored at -10 ^{o}C in a walk-in freezer. Spread, except the flaxseed oil spread, was prepared approximately 1 month prior to the start of the study. The flaxseed oil spread was prepared weekly so as to prevent oxidation of the flaxseed oil. The flaxseed oil spread was stored at -10 ^{o}C, whereas all other spread was stored at 7 ^{o}C. Except as outlined above, all food items were prepared

in the metabolic unit immediately prior to serving. All items were carefully weighted and prepared according to instructions outlined in the recipes. The recipes were similar to those used by Corner (1989). In some cases, modifications were made and some new recipes were added (see 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 taken for proximate analysis. Protein content of the diets was determined by a modified Kjeldahl method (AACC method 46-12, modified by Williams, 1973). The fat content was determined by the method of Bligh and Dyer (1959). The CHO content was derived by subtraction. Fatty acid composition of the fat extracted by the method of Bligh and Dyer (1959) was determined by gas chromatography. The fatty acids were methylated using sodium methoxide in methanol (Bannon et al, 1985). The fatty acid methyl esters were analyzed with a Hewlett-Packard (Model 5890) gas chromatograph. Samples obtained from replicate I were analyzed with a Durabond-225 capillary column, 30m x 0.25mm, film thickness 0.25 microns (J&W Scientific Inc.). Samples from replicate II were analyzed using a SP-2330 capillary column, 30m x 0.25mm, film thickness 0.2 microns (Supelco Inc.). Injector, detector

and column temperatures were: 250 °C, 250 °C and 210 °C, respectively. The carrier gas was helium. Peak areas were measured with a Hewlett-Packard HP3392A integrator-recorder.

3.6. PLASMA LIPID ANALYSIS

3.6.1. Plasma Lipid and Lipoprotein Analysis

Venous blood samples were taken from each subject following a 12-hour overnight fast on Days 1, 7, 16, 25, 34, 43 and 49 of each replicate. Plasma lipid and lipoprotein analyses were performed on fresh samples. Platelet poor plasma was prepared and analyzed for TC, LDL-C, HDL-C, VLDL-C and TG contents as described by Corner (1989). Excess platelet poor plasma was stored at -10 °C until required for use in the PL and CE fatty acid composition analyses.

3.6.2. Plasma PLs and CE Fatty Acid Composition Analysis

The analysis of plasma lipid fatty acid composition was performed on blood samples collected on Days 7, 25 and 43 of both replicates. The fatty acid composition of plasma PLs and CEs was determined by the methods described by Corner (1989) except for the following changes. Distilled chloroform was used in making up solvent mixtures for use in the thin-layer chromatographic separation of the PL fractions. The pre-coated thin layer plates used for CE analysis were run in a solvent system made up of petroleum ether/ethyl ether/glacial acetic acid (20/80/1;v/v/v) for

overnight prewashing. The plates were air dried following the washing. Just prior to use they were activated, then spotted and developed in a solvent system of petroleum ether/ethyl ether/glacial acetic acid (80/20/1;v/v/v).

All samples were methylated with sodium methoxide in methanol (Bannon et al, 1985). The fatty acid methyl esters were analyzed using the Hewlett-Packard (Model 5890) gas chromatograph equipped with a Durabond-225 capillary column as described above. Flow rates and temperatures were the same. Retention times of known standards were used in identifying fatty acids. Chromatograms of these standards are shown in Appendix 6. Pentadecaenoic acid (15:0) was used as an internal standard in all samples.

Methylation blanks were run regularly, at least one per set of methylation, during the CE and PL analyses. Also, a blank spot was scraped from every second thin-layer plate developed for PL analysis. These blank spots were methylated and subjected to gas chromatographic analysis (Thin layer chromatography (TLC) blank). The amounts of fatty acids found in each sample were then adjusted for contaminations present in either the methylating and/or the thin-layer procedures (Appendix 7).

3.7. STATISTICAL ANALYSIS

Statistical analyses were performed on the plasma lipid and lipoprotein data for Days 7, 25, 43 and 49 of both

replicates, and on the plasma PL and CE fatty acid data for Days 7, 25 and 43 of both replicates.

The experimental design of the study was a split plot within which was included a Latin square. The TC level of the subjects on Day 1 of replicate I, which were classified as either `high' or `low', was the treatment in the whole plot. Day and Subject were the two blocks in the latin square, Diet was the treatment.

Since there were two subjects, Subject 2 and Subject 9, completed only one-half of the study, the design was no longer balanced. Regression analysis (Steel and Torrie, 1980), where a balanced design is not necessary, was used to perform the analyses. Data were coded by the method of effect coding (Milliken and Johnson, 1984). F tests were performed for the estimated day effects and diet effects obtained from the regression analysis. Fatty acid levels in each lipid species were subjected to Pearson correlation analysis. All statistical analyses were performed using the SAS computer program (1984, 1986 SAS institute Inc., Cary, N.C.). All means reported herein have been adjusted for the presence of incomplete data.

A sample of data analysis is shown in Appendix 8. Sample calculation of adjusted means and the standard errors (SE) of the adjusted means are included in Appendix 9.

4. RESULTS

4.1. SUBJECTS

Subject 2 left after the completion of replicate I, so subject 9 was recruited for replicate II. This change was taken into account in the statistical analysis procedure. The results reported herein, therefore, were not confounded by the presence of a different subject in the second replicate.

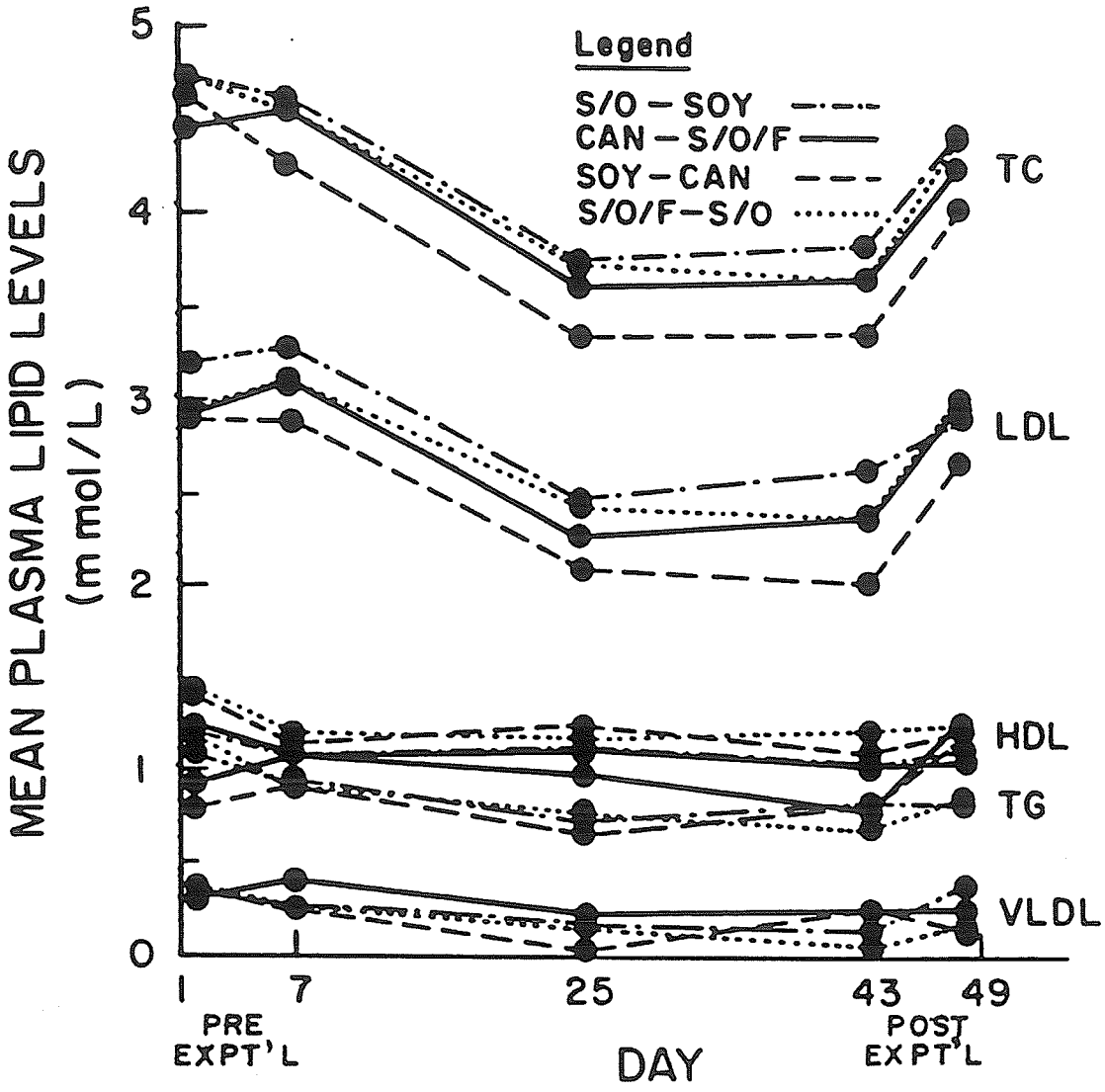
Subject compliance was monitored through weight changes and personal contact with the subjects. The subjects remained motivated throughout the study and their compliance was considered satisfactory.

Weight changes of the subjects were within the range of $\pm$ 1 kg, except in three cases; subject 1 lost 1.8 kg during replicate I, subject 4 lost 3.2 kg during replicate II and subject 5 gained 1.8 kg during replicate I.

4.2 Plasma Lipids and Lipoproteins

Changes in plasma lipid levels in response to the different diet sequences are shown in Figure 4. Results reported are means of the two replicates. Changing from the mixed fat diet to the experimental fat diets led to a decrease in the TC and LDL-C levels for all groups, regardless of the experimental diets involved. Changing the subjects to a different experimental fat at Day 25 did not produce any further change in these lipids. However, TC and

Figure 4. Mean Plasma Lipid Levels with Different Diets and Diet Sequences



LDL-C levels rose again when the subjects were returned to the mixed fat diet during the post-experimental period. The decrease in TC was due primarily to the decrease in LDL-C, although small decreases also occurred in VLDL-C. Changes in VLDL-C and TG followed more or less the same pattern described for TC and LDL-C, although the responses varied among the experimental diets. Dietary fat source had no effect on HDL-C levels. Figure 5 shows the pattern of change in mean plasma lipid levels during the different experimental periods when the data for all subjects in both replicates were combined.

As shown in Table 7, there were significant differences in the mean TC, LDL-C, VLDL-C and TG levels following the mixed fat diet and the experimental diets. No differences were found in the mean HDL-C levels. Mean TC level was 18% lower following the experimental diets than following the mixed fat diet. Mean LDL-C level was 22% lower, mean VLDL-C level was 41% lower and mean TG level was 22% lower following the experimental diets. In interpreting the results, however, one must bear in mind the fact that differences observed could be attributable to diet change, day effect or both.

The mean plasma lipid and lipoprotein levels following the different experimental fat sources are shown in Figure 6. No significant differences in plasma total or lipoprotein cholesterol levels were found among the

Figure 5. Changes in Mean Plasma Lipid Levels in Different Experimental Periods

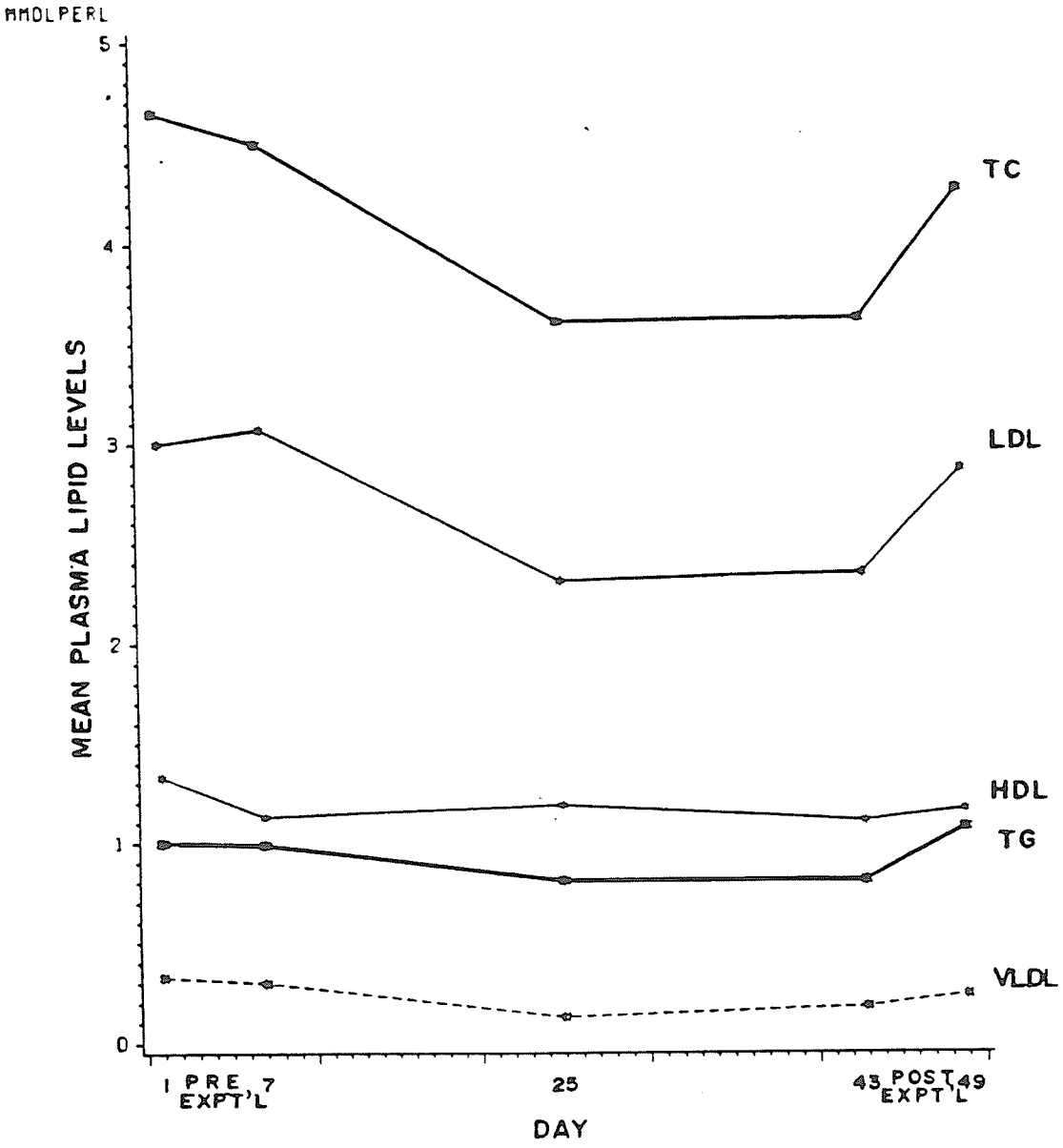
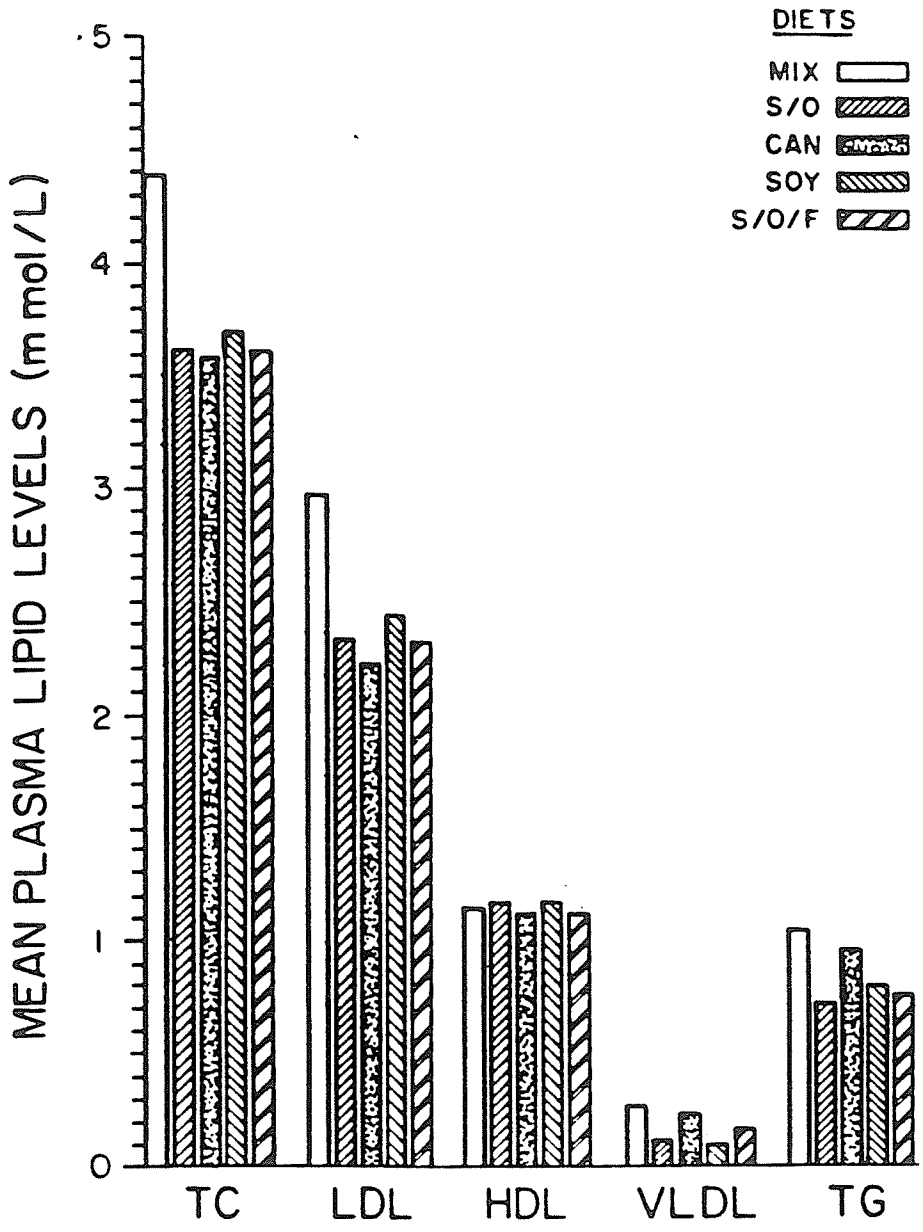


Table 7. Mean Plasma Lipid and Lipoprotein Levels Following the Mixed Fat and Experimental Fat Diets[1]

Plasma Lipid	Mixed Fat	Expt'l Fat	Difference
	(mmol/L)		
TC	4.40 ± 0.04	3.63 ± 0.04	-18%[2]
LDL	2.98 ± 0.05	2.34 ± 0.05	-22%[2]
HDL	1.15 ± 0.01	1.15 ± 0.01	0%
VLDL	0.27 ± 0.03	0.16 ± 0.03	-41%[3]
TG	1.04 ± 0.03	0.81 ± 0.03	-22%[2]

1 Values reported under the Mixed Fat period are mean values for D7 and D49 of both replicates of all subjects. Values reported under the experimental fat period are means of lipid values obtained on D25 and D43 of both replicates of all subjects.
All values are mean ± SE.
2 $p = 0.0001$.
3 $p = 0.0035$.

Figure 6. The Effects of Diets on Plasma Lipid Levels



experimental diets. Mean TG level, however, was significantly lower following the S/O and S/O/F diets than following the CAN diet (Table 8).

4.3. Plasma Phospholipid and Cholesterol Ester Fatty Acid Patterns

The fatty acid data for the plasma PC, PE, PPE and CEs were subjected to the same statistical analyses. Only small amounts of palmitic acid (PMA) and stearic acid (STEA) were detected in the PPE fraction, hence the data are not reported. Similarly, docosatetraenoic acid (DTA) and docosapentaenoic acid (DPA) levels were barely detectable in the CEs, so no data were reported for these fatty acids in CEs. Data for the n-6 and n-3 series of PUFA with chain lengths longer than eighteen also were pooled in an effort to see whether or not differences in the levels and ratio of LA and LNA in the diet had an effect on the overall level of the desaturated and elongated fatty acids in these series in plasma PLs and CEs.

4.3.1. Comparison Between the Mixed Fat and the Experimental Fat Diets

Statistical comparisons were made between the mixed fat and each of the experimental diets for each of the fatty acids in plasma PLs and CEs. As with the results observed in plasma lipids, any differences observed could be

Table 8. Mean Lipid and Lipoprotein Levels Following Different Experimental Diets[1]

Plasma Lipid	Experimental Fat Diets			
	S/O	CAN	SOY	S/O/F
	(mmol/L)			
TC	3.62±0.09[a2]	3.59±0.08[a]	3.70±0.08[a]	3.62±0.09[a]
LDL	2.34±0.09[a]	2.23±0.09[a]	2.44±0.09[a]	2.33±0.09[a]
HDL	1.17±0.02[a]	1.13±0.02[a]	1.17±0.02[a]	1.13±0.02[a]
VLDL	0.12±0.06[a]	0.24±0.05[a]	0.10±0.05[a]	0.17±0.06[a]
TG	0.73±0.06[a]	0.95±0.06[b]	0.79±0.06[ab]	0.76±0.06[a]

1 All values are mean ± SE. Plasma lipid data for individual subjects are shown in Appendix 9.
2 Means in the same row with the same letter do not differ ($p > 0.05$ level).

attributable to diet change, day effect or both.

Phosphatidylcholine (PC) (Table 9)

When compared to the mixed fat diet, the S/O diet resulted in significantly lower levels of PMA, STEA, LNA and EPA and significantly higher levels of OA, AA and pooled n-6 PUFA. The CAN diet, by contrast, was associated with a significantly lower level of PMA and significantly higher levels of OA, LNA, EPA, DPA and pooled n-3 PUFA than the mixed fat diet. The SOY diet, when compared with the mixed fat diet, was associated with significantly lower levels of OA, eicosatrienoic acid (ETA) and EPA and a significantly higher level of LA. The S/O/F diet was associated with significantly lower levels of OA, ETA, AA, DTA and pooled n-6 PUFA and significantly higher levels of STEA, LA, LNA and EPA than the mixed fat diet.

Phosphatidylethanolamine (PE) (Table 10)

As with the PC fraction, the S/O diet was associated with significantly lower levels of PMA and STEA and a significantly higher level of OA than the mixed fat diet. Changes associated with the CAN and SOY diets, again, bore considerable similarity with those observed in the PC fraction. The CAN diet, when compared to the mixed fat diet, was associated with significantly lower levels of PMA, STEA and DTA and significantly higher levels of OA, LNA, EPA

Table 9. Mean Plasma Phosphatidylcholine Fatty Acid Levels Following the Mixed Fat and the Experimental Diets[1]

Fatty Acid(s)[2]	Mixed	Diets S/O	CAN	SOY	S/O/F
		(Percent of total fatty acids)			
16:0	33.9±0.5	31.1±1.1[a3]	30.6±1.0[a]	32.5±1.0	32.6±1.1
18:0	15.7±0.2	15.1±0.3[a]	15.6±0.3	16.3±0.3	17.4±0.3[a]
18:1	13.1±0.2	15.1±0.4[a]	15.7±0.3[a]	9.3±0.3[a]	9.9±0.4[a]
18:2	22.7±0.3	23.3±0.6	21.8±0.5	27.9±0.5[a]	27.0±0.6[a]
18:3	0.3±0.02	0.2±0.04[a]	0.4±0.04[a]	0.3±0.04	0.7±0.04[a]
20:3	2.3±0.1	2.6±0.2	2.2±0.1	1.7±0.1[a]	1.6±0.2[a]
20:4	7.5±0.2	8.2±0.4[a]	7.9±0.3	7.9±0.3	6.6±0.4[a]
20:5	0.5±0.03	0.3±0.06[a]	0.9±0.06[a]	0.3±0.06[a]	0.7±0.06[a]
22:4	0.2±0.02	0.2±0.03	0.1±0.03	0.1±0.03	0.1±0.03[a]
22:5	0.6±0.03	0.5±0.07	0.7±0.06[a]	0.5±0.06	0.6±0.07
22:6	2.4±0.1	2.5±0.2	2.5±0.2	2.1±0.2	2.0±0.2
n-6[4]	9.9±0.2	11.0±0.5[a]	10.2±0.4	9.7±0.4	8.3±0.5[a]
n-3[5]	3.4±0.1	3.3±0.3	4.2±0.3[a]	3.0±0.3	3.3±0.3

1 Values reported under the Mixed Fat period are means of fatty acid values for all subjects on D7 of both replicates.
Plasma PC data for individual subjects are shown in Appendix 10. A sample fatty acid chromatogram for plasma PC is shown in Appendix 11.
All values are mean ± SE.

2 Carbon chain length:number of double bond.

3 Values in rows with a letter differ significantly ($p<0.05$) from the values for the mixed fat diet. p values for the statistical comparison of each experimental fat diet to the mixed fat diet are shown in Appendix 13.

4 n-6 = 20:3+20:4+22:4.

5 n-3 = 20:5+22:5+22:6.

Table 10. Mean Plasma Phosphatidylethanolamine Fatty Acid Levels Following the Mixed Fat and the Experimental Diets[1]

Fatty Acid(s)[2]	Mixed	Diets S/O	CAN	SOY	S/O/F
		(Percent of total fatty acids)			
16:0	12.9±0.3	11.2±0.7[a3]	10.3±0.6[a]	11.3±0.6[a]	10.4±0.7[a]
18:0	32.0±0.4	29.1±0.8[a]	28.9±0.7[a]	32.0±0.7	34.0±0.8[a]
18:1	14.2±0.4	18.3±0.7[a]	18.6±0.7[a]	11.8±0.7[a]	13.3±0.7
18:2	12.3±0.4	12.0±0.9	11.6±0.8	16.1±0.8[a]	15.1±0.9[a]
18:3	0.3±0.03	0.3±0.06	0.6±0.06[a]	0.5±0.06[a]	0.9±0.06[a]
20:3	1.3±0.03	1.3±0.06	1.3±0.06	1.0±0.06[a]	1.0±0.06[a]
20:4	18.4±0.5	19.0±1.0	17.8±0.9	18.3±0.9	16.5±1.0
20:5	0.5±0.04	0.4±0.08	0.9±0.07[a]	0.4±0.07[a]	0.8±0.08[a]
22:4	0.6±0.04	0.6±0.08	0.3±0.07[a]	0.5±0.07	0.5±0.08
22:5	1.1±0.1	1.0±0.1	1.3±0.1	1.2±0.1	1.5±0.1[a]
22:6	5.1±0.2	5.4±0.5	5.9±0.5	5.2±0.5	4.8±0.5
n-6[4]	20.3±0.5	20.9±1.1	19.5±1.0	20.0±1.0	18.0±1.1[a]
n-3[5]	6.7±0.3	6.7±0.7	8.1±0.6[a]	6.7±0.6	7.0±0.7

1 Values reported under the Mixed Fat period are means of fatty acid values for all subjects on D7 of both replicates.
Plasma PE data for individual subjects are shown in Appendix 13. A sample fatty acid chromatogram for plasma PE is shown in Appendix 14.
All values are mean ± SE.

2 Carbon chain length:number of double bond.

3 Values in rows with a letter differ significantly ($p<0.05$) from the values for the mixed fat diet. p values for the statistical comparison of each experimental diet to the mixed fat diet are shown in Appendix 16.

4 n-6 = 20:3+20:4+22:4.

5 n-3 = 20:5+22:5+22:6.

and pooled n-3 PUFA. The SOY diet was associated with significantly lower levels of PMA, OA, ETA and EPA and significantly higher levels of LA and LNA than the mixed fat diet. When compared to the mixed fat diet, the S/O/F diet was associated with significantly lower levels of PMA, ETA and pooled n-6 PUFA and significantly higher levels of STEA, LA, LNA, EPA and DPA than the mixed fat diet. Except for the PMA and DPA, the same pattern of change was also observed in the PC fraction.

Alkenylacyl Phosphatidylethanolamine (PPE) (Table 11)

As for the PC and PE fractions, the S/O diet was associated with a significantly higher level of OA than the mixed fat diet. As with the PC fraction, the S/O diet also was associated with a significantly lower level of EPA than the mixed fat diet. The significantly lower levels of ETA, DTA, DPA and pooled n-3 PUFA following the S/O diet, however, were found only in the PPE fraction. The CAN diet, when compared with the mixed fat diet, was associated with significantly higher levels of OA, LNA, EPA and n-3 PUFA, and significantly lower levels of LA, ETA and DTA. Similar increases in the n-3 PUFA also were observed in the PC and PE fractions, but the increase in DPA level found in the PC fraction was not found in the PPE. A significantly lower level of DTA also was observed in the PE fraction following the CAN diet than the mixed fat diet. As seen in

Table 11. Mean Plasma Alkenylacyl Phosphatidylethanolamine Fatty Acid Levels Following the Mixed Fat and the Experimental Diets[1]

Fatty Acid(s)[2]	Mixed	S/O	Diets CAN	SOY	S/O/F
		(Percent of total fatty acids)			
18:1	6.7±0.3	8.8±0.6[a3]	8.0±0.5[a]	5.4±0.5[a]	4.9±0.6[a]
18:2	14.4±0.5	14.5±1.1	12.1±1.0[a]	17.3±1.0[a]	18.5±1.1[a]
18:3	0.3±0.03	0.3±0.07	0.6±0.06[a]	0.5±0.06[a]	0.8±0.07[a]
20:3	2.4±0.06	2.1±0.1[a]	1.9±0.1[a]	1.4±0.1[a]	1.6±0.1[a]
20:4	47.9±0.6	47.7±1.2	46.8±1.1	46.7±1.1	45.2±1.2[a]
20:5	2.5±0.1	1.5±0.2[a]	3.6±0.2[a]	1.6±0.2[a]	3.1±0.2[a]
22:4	3.1±0.05	2.8±0.1[a]	2.5±0.1[a]	3.3±0.1	2.7±0.1[a]
22:5	6.1±0.1	5.2±0.3[a]	6.1±0.3	6.3±0.3	6.9±0.3[a]
22:6	14.5±0.3	14.4±0.5	15.5±0.5	15.8±0.5[a]	14.7±0.5
n-6[4]	53.3±0.6	52.5±1.2	51.2±1.1	51.3±1.1	49.4±1.2[a]
n-3[5]	23.0±0.4	21.0±0.8[a]	25.2±0.7[a]	23.6±0.7	24.6±0.8

1 Values reported under the Mixed Fat period are means of fatty acid values for all subjects on D7 of both replicates.
Plasma PPE data for individual subjects are shown in Appendix 16. A sample fatty acid chromatogram for plasma PPE is shown in Appendix 17.
All values are mean ± SE.
2 Carbon chain length:number of double bond.
3 Values in rows with a letter differ significantly ($p<0.05$) from the values for the mixed fat diet. p values for the statistical comparison of each experimental diet to the mixed fat diet are shown in Appendix 19.
4 n-6 = 20:3+20:4+22:4.
5 n-3 = 20:5+22:5+22:6.

the other two PL fractions, the SOY diet was associated with significantly lower levels of OA, ETA and EPA than the mixed fat diet. As with the PE fraction, significantly higher levels of LA and LNA were found following the SOY than the mixed fat diet. Surprisingly, the DHA level was significantly higher following the SOY than the mixed fat diet, the same change was not observed in the PC and PE species. As with the PC fraction, the S/O/F diet, when compared to the mixed fat diet, was associated with significantly lower levels of OA, ETA, AA, DTA and pooled n-6 PUFA. As with the PC and PE fractions, the S/O/F diet was associated with significantly higher levels of LA, LNA, EPA and DPA than the mixed fat diet, except that the effect of the diets on the DPA level was not found in the PC fraction.

Cholesteryl Esters (CEs) (Table 12)

The S/O diet was associated with a significantly higher level of OA and significantly lower levels of LNA, AA, EPA, n-6 PUFA and n-3 PUFA than the mixed fat diet. The higher level of OA following the S/O diet was similar to those found in the PL fractions. Differences in the LNA, EPA and n-3 PUFA levels also were similar to those observed in the PC and PPE species. As with the PC and PE, the PMA and STEA levels were significantly lower following the CAN diet than the mixed fat diet; and as with all the PL fractions, the levels of OA, LNA, EPA and n-3 PUFA (EPA + DHA) were

Table 12. Mean Plasma Cholesteryl Ester Fatty Acid Levels Following the Mixed Fat and the Experimental Diets[1]

Fatty Acid(s)[2]	Mixed	Diets S/O	CAN	SOY	S/O/F
		(Percent of total fatty acids)			
16:0	10.3±0.2	10.6±0.5	9.2±0.4[a3]	9.7±0.4	8.8±0.5[a]
18:0	0.7±0.05	0.6±0.1	0.4±0.1[a]	0.4±0.1[a]	0.6±0.1
18:1	19.3±0.2	22.4±0.4[a]	21.2±0.3[a]	11.7±0.3[a]	13.5±0.4[a]
18:2	57.7±0.3	56.5±0.6	57.0±0.6	68.1±0.6[a]	66.0±0.6[a]
18:3	0.6±0.04	0.3±0.07[a]	1.1±0.07[a]	0.8±0.07[a]	1.9±0.07[a]
20:3	0.4±0.04	0.4±0.09	0.5±0.08	0.3±0.08	0.3±0.09
20:4	7.5±0.07	7.1±0.2[a]	7.2±0.1	7.0±0.1[a]	6.2±0.2[a]
20:5	0.6±0.06	0.3±0.1[a]	1.0±0.1[a]	0.4±0.1	1.0±0.1[a]
22:6	0.5±0.04	0.4±0.07	0.5±0.07	0.5±0.07	0.4±0.07
n-6[4]	7.9±0.07	7.5±0.2[a]	7.7±0.1	7.3±0.1[a]	6.6±0.2[a]
n-3[5]	1.1±0.08	0.6±0.2[a]	1.5±0.2[a]	0.9±0.2	1.4±0.2

1 Values reported under the Mixed Fat period are means of fatty acid values for all subjects on D7 of both replicates.
Plasma CE data for individual subjects are shown in Appendix 19. A sample fatty acid chromatogram for plasma CE is shown in Appendix 20.
All values are mean ± SE.

2 Carbon chain length:number of double bond.

3 Values in rows with a letter differ significantly ($p<0.05$) from the values for the mixed fat diet. p values for the statistical comparison of each experimental fat diet to the mixed fat diet are shown in Appendix 22.

4 n-6 = 20:3+20:4.

5 n-3 = 20:5+22:6.

significantly higher following the CAN than the mixed fat diet. The SOY diet, when compared with the mixed fat diet, was associated with significantly lower levels of STEA, OA, AA and n-6 PUFA (ETA + AA), and significantly higher levels of LA and LNA. Similar differences between the SOY and mixed fat diets for OA, LA and LNA levels also were found in the PL fractions. The S/O/F diet was associated with significantly lower levels of PMA, OA, AA and n-6 PUFA (ETA + AA) and significantly higher levels of LA, LNA and EPA than the mixed fat diet. Again, similar patterns following the S/O/F and mixed fat diets were observed for the PL fractions.

4.3.2 Comparison Among Different Experimental Fat Sources

Phosphatidylcholine (PC) (Table 13)

No significant differences in PMA level of the PC fraction were found among the experimental fat diets. The SOY and the S/O/F diets were associated with significantly higher levels of STEA, although the level following the SOY diet did not differ from that following the CAN diet. These findings could be due to the fact that dietary intake of STEA was higher with the SOY and the S/O/F diets. The S/O and the CAN diets were associated with significantly higher levels of OA, whereas the SOY and the S/O/F diets were associated with significantly higher levels of LA. LNA levels in plasma PC also reflected the fatty acid

Table 13. Mean Plasma Phosphatidylcholine Fatty Acid Levels Following Different Experimental Diets[1]

Fatty Acid(s)[2]	Experimental Diets S/O	CAN	SOY	S/O/F
	(Percent of total fatty acids)			
16:0	31.1±1.1[a3]	30.6±1.0[a]	32.5±1.0[a]	32.6±1.1[a]
18:0	15.1±0.3[a]	15.6±0.3[ab]	16.3±0.3[b]	17.4±0.3[c]
18:1	15.1±0.4[a]	15.7±0.3[a]	9.3±0.3[b]	9.9±0.4[b]
18:2	23.3±0.6[a]	21.8±0.5[a]	27.9±0.5[b]	27.0±0.6[b]
18:3	0.2±0.04[a]	0.4±0.04[b]	0.3±0.04[b]	0.7±0.04[c]
20:3	2.6±0.2[a]	2.2±0.1[a]	1.7±0.1[b]	1.6±0.2[b]
20:4	8.2±0.4[a]	7.9±0.3[a]	7.9±0.3[a]	6.6±0.4[b]
20:5	0.3±0.06[a]	0.9±0.06[b]	0.3±0.06[a]	0.7±0.06[c]
22:4	0.2±0.03[a]	0.1±0.03[b]	0.1±0.03[b]	0.1±0.03[b]
22:5	0.5±0.07[a]	0.7±0.06[b]	0.5±0.06[a]	0.6±0.07[ab]
22:6	2.5±0.2[a]	2.5±0.2[a]	2.1±0.2[a]	2.0±0.2[a]
n-6[4]	11.0±0.5[a]	10.2±0.4[ab]	9.7±0.4[b]	8.3±0.5[c]
n-3[5]	3.3±0.3[a]	4.2±0.3[b]	3.0±0.3[a]	3.3±0.3[a]

1 All values are mean ± SE.
2 Carbon chain length:number of double bond.
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 23.
4 n-6 = 20:3+20:4+22:4.
5 n-3 = 20:5+22:5+22:6.

composition of the diets. The CAN and the SOY diets led to significantly higher levels of LNA than the S/O diet while the level of LNA following the S/O/F diet was significantly higher than following any of the other diets.

The level of long chain PUFA also was affected, to some degree, by the fatty acid composition of the diets. The level of ETA was significantly lower following the SOY and S/O/F diets than following the other two diets. The level of AA was significantly lower following the S/O/F diet while the level of DTA was significantly lower following the diets containing LNA (i.e., CAN, SOY and S/O/F). The magnitude of the latter, however, was very small (0.1%). The CAN and S/O/F diets were associated with significantly higher levels of EPA than the S/O and SOY diets with the level following the CAN diet being significantly higher than following the S/O/F diet. Similarly the DPA level was significantly higher following the CAN diet than following the S/O and SOY diets. On the other hand, there were no differences in DHA level among the different diets. The SOY and S/O/F diets were associated with significantly lower levels of total n-6 PUFA, although the difference between the CAN and SOY diets was not significant. By contrast, only the CAN diet resulted in significantly higher level of total n-3 PUFA in the PC fraction.

Phosphatidylethanolamine (PE) (Table 14)

As with the PC fraction, no differences in PMA level were found among the experimental diets. Similarly the SOY and the S/O/F diets were associated with significantly higher levels of STEA and LA while the S/O and the CAN diets were associated with significantly higher levels of OA. As with the PC fraction, the CAN and SOY diets led to significantly higher LNA levels than the S/O diet while the level was significantly higher following the S/O/F diet than any of the other three diets.

The level of ETA in the PE fraction also was significantly lower following the SOY and S/O/F diets. The level of AA, however, did not differ among the experimental diets and the level of DTA was significantly lower following the CAN diet than for the S/O diet. Unlike the effect for the PC fraction, there were no differences in n-6 PUFA level among the diets. As with the PC fraction, the level of EPA was significantly higher following the CAN and S/O/F diets. However, with the PE fraction, a significantly higher level of DPA was observed following the S/O/F diet than following the S/O diet. As with the PC fraction, no differences were observed in DHA among the different diets. Similarly, no differences were observed in pooled n-3 PUFA levels.

Alkenylacylphosphatidylethanolamine (PPE) (Table 15)

As with the other PL fractions, the S/O and the CAN

Table 14. Mean Plasma Phosphatidylethanolamine Fatty Acid Levels Following Different Experimental Diets[1]

Fatty Acid(s)[2]	Experimental Diets S/O	CAN	SOY	S/O/F
	(Percent of total fatty acids)			
16:0	11.2±0.7[a3]	10.3±0.6[a]	11.3±0.6[a]	10.4±0.7[a]
18:0	29.1±0.8[a]	28.9±0.7[a]	32.0±0.7[b]	34.0±0.8[b]
18:1	18.3±0.7[a]	18.6±0.7[a]	11.8±0.7[b]	13.3±0.7[b]
18:2	12.0±0.9[a]	11.6±0.8[a]	16.1±0.8[b]	15.1±0.9[b]
18:3	0.3±0.06[a]	0.6±0.06[b]	0.5±0.06[b]	0.9±0.06[c]
20:3	1.3±0.06[a]	1.3±0.06[a]	1.0±0.06[b]	1.0±0.06[b]
20:4	19.0±1.0[a]	17.8±0.9[a]	18.3±0.9[a]	16.5±1.0[a]
20:5	0.4±0.08[a]	0.9±0.07[b]	0.4±0.07[a]	0.8±0.08[b]
22:4	0.6±0.08[a]	0.3±0.07[b]	0.5±0.07[ab]	0.5±0.08[ab]
22:5	1.0±0.1[a]	1.3±0.1[ab]	1.2±0.1[ab]	1.5±0.1[b]
22:6	5.4±0.5[a]	5.9±0.5[a]	5.2±0.5[a]	4.8±0.5[a]
n-6[4]	20.9±1.1[a]	19.5±1.0[a]	20.0±1.0[a]	18.0±1.1[a]
n-3[5]	6.7±0.7[a]	8.1±0.6[a]	6.7±0.6[a]	7.0±0.7[a]

1 All values are mean ± SE.
2 Carbon chain length:number of double bond.
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 24.
4 n-6 = 20:3+20:4+22:4.
5 n-3 = 20:5+22:5+22:6.

Table 15. Mean Plasma Alkenylacyl Phosphatidylethanolamine Fatty Acid Levels Following Different Experimental Diets[1]

Fatty Acid(s)[2]	Experimental Diets S/O	CAN	SOY	S/O/F
	(Percent of total fatty acids)			
18:1	8.8±0.6[a3]	8.0±0.5[a]	5.4±0.5[b]	4.9±0.6[b]
18:2	14.5±1.1[ab]	12.1±1.0[a]	17.3±1.0[bc]	18.5±1.1[c]
18:3	0.3±0.07[a]	0.6±0.06[bc]	0.5±0.06[b]	0.8±0.07[c]
20:3	2.1±0.1[a]	1.9±0.1[ab]	1.4±0.1[c]	1.6±0.1[bc]
20:4	47.7±1.2[a]	46.8±1.1[a]	46.7±1.1[a]	45.2±1.2[a]
20:5	1.5±0.2[a]	3.6±0.2[b]	1.6±0.2[a]	3.1±0.2[b]
22:4	2.8±0.1[a]	2.5±0.1[a]	3.3±0.1[b]	2.7±0.1[a]
22:5	5.2±0.3[a]	6.1±0.3[b]	6.3±0.3[b]	6.9±0.3[b]
22:6	14.4±0.5[a]	15.5±0.5[a]	15.8±0.5[a]	14.7±0.5[a]
n-6[4]	52.5±1.2[a]	51.2±1.1[a]	51.3±1.1[a]	49.4±1.2[a]
n-3[5]	21.0±0.8[a]	25.2±0.7[b]	23.6±0.7[b]	24.6±0.8[b]

1 All values are mean ± SE.
2 Carbon chain length:number of double bond.
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 25.
4 n-6 = 20:3+20:4+22:4.
5 n-3 = 20:5+22:5+22:6.

diets were associated with significantly higher levels of OA and the SOY and the S/O/F diets with significantly higher levels of LA although the level following the SOY diet was not significantly higher than following the S/O diet. The CAN, SOY and S/O/F diets again resulted in significantly higher levels of LNA with the highest level associated with the S/O/F diet.

The SOY and S/O/F diets were associated with significantly lower levels of ETA, but the levels for the CAN and the S/O/F diets did not differ significantly. As with the PE fraction, no significant differences were found in AA and n-6 PUFA content among the diets but unlike the PC and PE fractions, a significantly higher level of DTA was observed following the SOY diet. The CAN and the S/O/F diets again were associated with significantly higher levels of EPA and the level of DPA was significantly higher following all of the diets containing LNA. As with the PC and PE fractions, experimental fat source had no effect on the level of DHA. However, the pooled n-3 PUFA level was significantly higher following all three diets containing LNA.

Cholesteryl Esters (CEs) (Table 16)

The level of PMA in the CE fraction, unlike those in the PL, was significantly lower following the CAN and S/O/F diets than following the S/O diet. These differences might

Table 16. Mean Plasma Cholesteryl Ester Fatty Acid Levels Following Different Experimental Diets[1]

Fatty Acid(s)[2]	Experimental Diets S/O	CAN	SOY	S/O/F
	(Percent of total fatty acids)			
16:0	10.6±0.5[a3]	9.2±0.4[b]	9.7±0.4[ab]	8.8±0.5[b]
18:0	0.6±0.1[a]	0.4±0.1[a]	0.4±0.1[a]	0.6±0.1[a]
18:1	22.4±0.4[a]	21.2±0.3[b]	11.7±0.3[c]	13.5±0.4[d]
18:2	56.5±0.6[a]	57.0±0.6[a]	68.1±0.6[b]	66.0±0.6[c]
18:3	0.3±0.07[a]	1.1±0.07[b]	0.8±0.07[c]	1.9±0.07[d]
20:3	0.4±0.09[ab]	0.5±0.08[a]	0.3±0.08[b]	0.3±0.09[ab]
20:4	7.1±0.2[a]	7.2±0.1[a]	7.0±0.1[a]	6.2±0.2[b]
20:5	0.3±0.1[a]	1.0±0.1[b]	0.4±0.1[a]	1.0±0.1[b]
22:6	0.4±0.07[a]	0.5±0.07[a]	0.5±0.07[a]	0.4±0.07[a]
20:3+20:4	7.5±0.2[ab]	7.7±0.1[a]	7.3±0.1[b]	6.6±0.2[c]
20:5+22:6	0.6±0.2[a]	1.5±0.2[b]	0.9±0.2[a]	1.4±0.2[b]

1 All values are mean ± SE.
2 Carbon chain length:number of double bond.
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.

have been due to the lower PMA intakes associated with the CAN and S/O/F diets (Appendix 3). If this was the case, however, such responses would have also been expected to occur in the PL fractions, particularly the PC fraction. Dietary fat source had no effect on the level of STEA in the CEs. As with the PLs, the S/O and CAN diets were associated with significantly higher levels of OA whereas the SOY and S/O/F diets were associated with significantly higher levels of LA. Significantly higher levels of LNA were observed when the subjects were fed the diets containing LNA; progressively higher levels were observed following the SOY, CAN and S/O/F diets, respectively.

The level of ETA was significantly lower following the SOY diet than following the CAN diet. However, the actual difference was small due to the relatively low level of ETA present in the CE fraction. As with the PC fraction, the S/O/F diet was associated with a significantly lower level of AA. Similarly, the SOY and the S/O/F diets were associated with significantly lower levels of pooled n-6 PUFA (ETA and AA), but unlike the levels in the PC fraction, the level was highest following the CAN diet and there was no difference between the S/O and SOY diets. As with the PLs, the CAN and S/O/F diets were associated with significantly higher levels of EPA. Similarly, there were no differences in DHA level among the diets. The levels of pooled n-3 PUFA (EPA and DHA) also were higher following the

CAN and S/O/F diets than following the other two diets.

4.3.3. Correlation Analysis of Plasma Phospholipid and Cholesterol Ester Fatty Acids (Table 17-20)

The level of LA was positively correlated with the level of LNA for all of the PL fractions and the CE. By contrast, there was a negative correlation between the level of LA and all long chain PUFA, although the degree of correlation was significant ($p<0.05$) only with: ETA in all the PL species; AA in the PE, PPE and CEs; DTA in the PE; and EPA in the PE and PPE fractions. The level of LNA also was negatively correlated with the level of: ETA in the PL fractions; AA in PE and PPE; and DTA in PC and PE. It was positively correlated with the level of EPA in PC and CEs. The level of ETA was positively correlated with the levels of: AA in the PC and PE fractions and DTA acid in the PC fraction. The level of AA was positively correlated with the level of EPA in the PC fraction; and the levels of DTA and DPA in the PC and PE fractions. The level of EPA was positively correlated with the level of DPA in all PL fractions and the level of DTA was positively correlated with the level of DPA in PE and PPE fractions.

4.4. Summary

The experimental fat diets, which differed in unsaturated fatty acid composition, resulted in similar

Table 17. Results of Correlation Analysis of Plasma Phosphatidylcholine Fatty Acids

	Pearson correlation coefficient/Prob						
Fatty Acid[1]	18:2	18:3	20:3	20:4	20:5	22:4	22:5
18:2	/	0.33 0.02	-0.29 0.05	-0.23 0.12	-0.20 0.18	0.00 0.99	-0.08 0.59
18:3	0.33 0.02	/	-0.47 0.0008	-0.16 0.28	0.40 0.005	-0.38 0.008	0.09 0.57
20:3	-0.29 0.05	-0.47 0.0008	/	0.63 0.0001	0.18 0.22	0.44 0.002	0.19 0.20
20:4	-0.23 0.12	-0.16 0.28	0.63 0.0001	/	0.40 0.005	0.39 0.007	0.45 0.002
20:5	-0.20 0.18	0.40 0.005	0.18 0.22	0.40 0.005	/	-0.15 0.30	0.54 0.0001
22:4	0.00 0.99	-0.38 0.008	0.44 0.002	0.39 0.007	-0.15 0.30	/	0.26 0.07
22:5	-0.08 0.59	0.09 0.57	0.19 0.20	0.45 0.002	0.54 0.0001	0.26 0.07	/

1 Carbon chain length:number of double bond.

Table 18. Results of Correlation Analysis of Plasma Phosphatidylethanolamine Fatty Acids

	Pearson correlation coefficient/Prob						
Fatty Acid[1]	18:2	18:3	20:3	20:4	20:5	22:4	22:5
18:2	/	0.49 0.0005	-0.48 0.0006	-0.55 0.0001	-0.30 0.04	-0.33 0.02	-0.24 0.09
18:3	0.49 0.0005	/	-0.48 0.0006	-0.44 0.002	0.27 0.06	-0.29 0.04	0.18 0.22
20:3	-0.48 0.0006	-0.48 0.0006	/	0.54 0.0001	0.29 0.05	0.15 0.30	0.16 0.27
20:4	-0.55 0.0001	-0.44 0.002	0.54 0.0001	/	0.27 0.06	0.64 0.0001	0.49 0.0004
20:5	-0.30 0.04	0.27 0.06	0.29 0.05	0.27 0.06	/	-0.02 0.91	0.74 0.0001
22:4	-0.33 0.02	-0.29 0.04	0.15 0.30	0.64 0.0001	-0.02 0.91	/	0.37 0.009
22:5	-0.24 0.09	0.18 0.22	0.16 0.27	0.49 0.0004	0.74 0.0001	0.37 0.009	/

1 Carbon chain length:number of double bond.

Table 19. Results of Correlation Analysis of Plasma Alkenylacyl Phosphatidylethanolamine Fatty Acids

	Pearson correlation coefficient/Prob						
Fatty Acid[1]	18:2	18:3	20:3	20:4	20:5	22:4	22:5
18:2	/	0.44 0.002	-0.32 0.03	-0.72 0.0001	-0.33 0.02	-0.18 0.23	-0.23 0.11
18:3	0.44 0.002	/	-0.57 0.0001	-0.53 0.0001	0.00 0.98	-0.26 0.07	-0.05 0.75
20:3	-0.32 0.03	-0.57 0.0001	/	0.20 0.18	0.12 0.43	0.01 0.93	-0.09 0.55
20:4	-0.72 0.0001	-0.53 0.0001	0.20 0.18	/	0.17 0.25	0.09 0.54	0.11 0.48
20:5	-0.33 0.02	0.00 0.98	0.12 0.43	0.17 0.25	/	-0.22 0.14	0.43 0.002
22:4	-0.18 0.22	-0.26 0.07	0.01 0.93	0.09 0.54	-0.22 0.14	/	0.57 0.0001
22:5	-0.23 0.11	-0.05 0.75	-0.09 0.55	0.11 0.48	0.43 0.002	0.57 0.0001	/

1 Carbon chain length:number of double bond.

Table 20. Results of Correlation Analysis of Plasma Cholesteryl Ester Fatty Acids

	Pearson correlation coefficient/Prob				
Fatty Acid[1]	18:2	18:3	20:3	20:4	20:5
18:2	/	0.51 0.0002	-0.24 0.10	-0.58 0.0001	-0.20 0.17
18:3	0.51 0.0002	/	0.14 0.33	-0.27 0.07	0.46 0.001
20:3	-0.24 0.10	0.14 0.33	/	0.10 0.52	0.24 0.11
20:4	-0.58 0.0001	-0.27 0.07	0.10 0.52	/	0.16 0.27
20:5	-0.20 0.17	0.46 0.001	0.24 0.11	0.16 0.27	/

1 Carbon chain length:number of double bond.

decreases in plasma TC, LDL-C and VLDL-C. The decrease in TC level associated with the experimental diets was due primarily to the decrease in the LDL-C level.

The mixed fat diet contained higher amounts of PMA and STEA than the experimental diets. Corresponding changes, however, were not seen in the fatty acid patterns of plasma PLs and CEs when the subjects were switched from the mixed to the experimental diets. By contrast, OA, LA and LNA levels in the plasma PLs and CEs following the consumption of the mixed fat and experimental diets reflected closely the fatty acid composition of the diets. Yet, the magnitude of change observed in plasma fatty acids was not comparable to that associated with dietary intakes. In general, the following differences in fatty acid patterns of the PLs and CEs were observed following the mixed fat and experimental diets: the S/O diet was associated with significantly higher levels of OA and significantly lower levels of PMA, STEA, LNA, EPA and pooled n-3 PUFA than the mixed fat diet; the CAN diet was associated with significantly lower levels of PMA, STEA and DTA and significantly higher levels of OA, LNA, EPA and n-3 PUFA than the mixed fat diet; the SOY diet was associated with significantly higher levels of LA and LNA and significantly lower levels of OA, ETA, and EPA than the mixed fat diet; and the S/O/F diet was associated with significantly higher levels of STEA, LA, LNA, EPA and DPA and significantly lower levels of PMA, OA, ETA, AA, DTA and

pooled n-6 PUFA than the mixed fat diet.

Similar changes also were observed when comparisons were made among the experimental diets. For fatty acids with chain lengths shorter than 20 carbons, plasma PL and CE fatty acid levels following the different experimental fat diets reflected closely the fatty acid composition of the diets. Diets which contained high amounts of OA (the CAN and S/O diets), LA (the SOY and S/O/F diets) and LNA (the CAN, SOY and S/O/F diets) were consistently associated with significantly higher levels of OA, LA and LNA, respectively, in plasma lipids. The SOY and S/O/F diets were frequently associated with significantly lower levels of ETA in plasma lipids. The S/O/F diet also was frequently associated with significantly lower levels of AA, whereas the CAN diet was associated with significantly lower levels of DTA. Both the CAN and S/O/F diets were consistently associated with significantly higher levels of EPA in plasma lipids. These two diets were also frequently associated with significantly higher levels of DPA in the plasma lipids. Dietary fat source had no effect on plasma DHA levels. The SOY and S/O/F diets were frequently associated with significantly lower levels of pooled n-6 PUFA, whereas the CAN and S/O/F diets were frequently associated with significantly higher levels of pooled n-3 PUFA.

The level of LA in the plasma was negatively correlated to the levels of ETA, AA, DTA and EPA in most PL and CE

fractions and positively correlated with the level of LNA in all the PL fractions and CEs, whereas plasma LNA was negatively correlated with ETA, AA and DTA and positively correlated to the level of EPA in most plasma lipid fractions.

5. DISCUSSION

5.1. PLASMA LIPID AND LIPOPROTEIN PATTERNS

The experimental diets used in tne present study contained similar amounts of SFA, which were about half the amount present in the mixed fat diet, but they differed in OA, LA and LNA composition. Mean plasma TC, LDL-C and VLDL-C levels were significantly lower, 18%, 22% and 41%, respectively, following the experimental fat diets than the mixed fat diet. The hypercholesterolemic nature of SFA has been well documented (Keys et al, 1957, Hegsted et al, 1965, Keys, 1970, Spady and Dietschy, 1988). Given the higher SFA content of the mixed fat diet, the different plasma cholesterol patterns observed for the mixed fat and the experimental diets is not surprising. However, the unsaturated fatty acids present in the experimental diets might have contributed to the cholesterol-lowering effects associated with these diets as well. The hypocholesterolemic effect of OA and LA has been repeatedly demonstrated in metabolic studies (Shepherd et al, 1978; Vega et al, 1982; Cortese et al, 1983; Mattson & Grundy, 1985; Mensink & Katan, 1987; Baggio et al, 1988; McDonald, et al, 1989). Contrasting evidence regarding the hypocholesterolemic effect of OA had been reported in earlier studies by Keys et al (1957) and Hegsted et al (1965). Changes in OA intake in some of these studies were always accompanied by changes in total fat content (Keys et

al, 1958; Keys et al, 1965) or SFA level (Keys et al, 1957) of the diets. Also, only a few levels of monounsaturates were tested in these studies (Keys et al, 1957; Hegsted et al, 1965). All these factors might have masked the cholesterol-lowering effect of OA to some degree. Keys et al (1965) and Lasserre et al (1985) found that a rapeseed oil-rich diet had a similar cholesterol-lowering effect as LA-rich or low-fat diets whereas only a moderate lowering-effect of LNA on plasma TC was showed in a long term diet study (Renaud et al, 1986). Studies by Mest et al (1983) and Sanders & Roshanai (1983) with linseed oil supplements, on the other hand, found that LNA had no effect on plasma lipid metabolism.

The mixed fat and experimental diets also differed in their cholesterol levels. Nevertheless, studies have shown that dietary cholesterol has a negligible effect on plasma cholesterol levels; it has been estimated that a decrease of 100 mg/day in cholesterol intake will lower plasma total cholesterol level by about 0.1 mmol/L (Hegsted et al, 1965; Grundy, 1986).

The question of what mechanisms are responsible for the cholesterol-lowering effects of diets rich in unsaturated fatty acids and low in SFA has not been settled. Results obtained from the present study cannot provide a direct answer to the above question, but they do provide some clues to the unsolved puzzle.

Diets with different unsaturated fatty acid composition were found to have similar cholesterol-lowering effects in the present study. This observation suggests that unsaturated fatty acids may exert their effects on plasma cholesterol through a common mechanism, possibly one that is related to the unsaturated nature of these fatty acids. Strong evidence has been provided by Spady & Dietschy (1988) for an effect of unsaturated fat in studies with hamsters fed diets containing different amounts of dietary cholesterol. They found that saturated fatty acids (coconut oil) augmented the suppressive effect of dietary cholesterol on hepatic LDL receptor activity whereas unsaturated fatty acids (olive oil or safflower oil) reduced the suppressive effect of dietary cholesterol on receptor activity. Other studies also have shown high OA or LA intakes lead to significant increases in the fractional catabolic rates of the LDL particles (International Collaborative Study Group, 1986; Shepherd et al, 1980). However, contrary results have been reported. Cortese et al (1983) found a lower rate of LDL synthesis with no changes in the fractional catabolic rate of the lipoprotein in response to the feeding of a sunflower oil-rich diet to a group of hypercholesterolemic subjects.

The unsaturated fatty acids may lower plasma cholesterol simply by replacing SFA, the hypercholesterolemic fatty acids, in the diet. Although

Grundy (1987) attributed the cholesterol-lowering effects of OA and LA to their replacement of SFA in the diet, Keys et al (1957) and Hegsted et al (1965) suggested that PUFA have a cholesterol-lowering effect beyond that associated with the replacement of SFA in the diet. A regression equation developed from data obtained in a series of metabolic studies (Keys et al, 1957) indicated that SFA were twice as effective in raising blood cholesterol levels as LA was in lowering them. The mechanisms through which these effects were mediated, however, were not studied by Grundy (1987), Keys et al (1957) and Hegsted et al (1965). In the study by Spady & Dietschy (1988), mentioned above, the addition of safflower oil alone to a control diet was associated with increased hepatic LDL clearance, indicating that LA may have an effect on LDL receptor activity other than that due simply to replacing the SFA in the diet.

Unsaturated fatty acids also may increase LDL lipid phase fluidity, by changing the fatty acid composition of the LDL particles, and thus enhance the LDL receptor mediated removal of cholesterol from circulation (Soutar, 1978; Paul et al, 1980). Berlin et al (1987) were able to demonstrate a moderate increase in LDL fluidity by feeding subjects a LA-rich diet. LA also has been shown to be capable of reducing the availability of precursor for LDL-C synthesis and enhancing the removal of cholesterol through increased excretion of bile acid (Nestel, 1987). Whether OA

and LNA would also affect plasma cholesterol metabolism through these mechanisms is not known.

The reductions in TC, LDL-C and VLDL-C induced by the experimental diets in the present study were comparable to those observed in metabolic studies which were similar in nature to the present study. Metabolic studies in which subjects were fed OA- and/or LA-rich diets for variable lengths of time were associated with significant decreases: in TC, ranged from 9-26%; in LDL-C, from 12-37%; and in VLDL, from 0-33% (Shepherd et al, 1978; Vega et al, 1982; Becker et al, 1983; Cortese et al, 1983; Mattson and Grundy, 1985; Mensink and Katan, 1987; Baggio et al, 1988). The degree of reduction in plasma cholesterol level observed in these studies probably varied because of differences in the type of diet used, the number and type of subjects, as well as the duration of the studies. A metabolic study conducted in our laboratory (McDonald et al, 1989) showed that both sunflower oil- and canola oil-rich diets lowered TC and LDL-C by 18% and 23%, respectively. These results were similar to those observed in the present study, although no changes on VLDL-C levels were detected in the earlier study (McDonald et al, 1989). These results suggest the pattern and magnitude of change observed in metabolic studies depends to a large extent on the design and methodology employed in the studies.

The effect of diets high in unsaturated fatty acids,

especially LA, on plasma HDL-C level has been of increasing concern, due to the inverse relationship between HDL-C level and the incidence of CHD (Castelli et al, 1986). Indeed, LA-rich diets have been shown to lower HDL-C levels in some metabolic studies (Shepherd et al, 1978; Shepherd et al, 1980; Mattson & Grundy, 1985; Sirtorri et al, 1986). Changing from the mixed fat diet to the experimental diets in the present study did not lead to any significant changes in the HDL-C level. This lack of effect may be due to the relatively low LA content and low P/S ratios of the experimental diets in the present study compared to the levels used in the studies in which an HDL-C-lowering effect of LA has been observed.

The S/O and S/O/F diets were associated with significantly lower TG levels than the CAN diet. Although the TG-lowering effect of dietary EPA, has been shown (Herold and Kinsella, 1986), the effect of LNA on plasma TG has been found to be negligible (Mest et al, 1983, Sanders & Roshanai, 1983; Renaud et al, 1986). Responses of plasma TG levels to a variety of vegetable oils have been reported by several investigators (Shepherd et al, 1978; Vega et al, 1982; Becker et al, 1983; Harris et al, 1983; Baggio et al, 1988), but no consistent effect was evident. To what extent differences in the fatty acid composition of the S/O, CAN and S/O/F diets accounted for the results found in plasma TG metabolism is not clear.

Due to the well established relationship between blood cholesterol level and risk of CHD, the experimental fats used in the present study would be expected to have a protective effect on CHD through their influence on plasma cholesterol levels, particularly the LDL-C. However, the mechanisms through which dietary unsaturated fatty acids lower plasma cholesterol levels remain to be defined.

5.2 PLASMA PHOSPHOLIPID AND CHOLESTERYL ESTER FATTY ACID PATTERNS

Levels of OA, LA and LNA in plasma PLs and CEs following the consumption of the experimental diets reflected closely the OA, LA and LNA composition of the diets, indicating that dietary fat source has an influence on plasma lipid fatty acid composition. It should be noted, however, that the level and the magnitude of change in LNA were small. Similar influences of dietary fat source on the fatty acid composition of plasma PLs and CEs have been reported in other studies (Zollner et al, 1979; Ferretti et al, 1985; Adam et al, 1986; Renaud et al, 1986). Increases in LNA intake have been reported to be associated with reduced plasma LA levels (Mest et al, 1983; Budowski et al, 1984). The lower LA levels were believed to be due to preferential incorporation of LNA into plasma lipids (Budowski et al, 1984). Preferential incorporation of LNA, however, was not observed in the present study. No

differences were found in plasma LA levels following the S/O and CAN diets, although the latter was higher in LNA content. Similarly, the SOY and S/O/F diets, which differed in their LNA contents, did not differ in their effects on plasma LA.

Although changes in the levels of fatty acids in plasma PLs and CEs were observed in response to changes in the fatty acid composition of the diets in the present study, the magnitude of the change in plasma fatty acid levels was small compared to the changes in dietary fatty acid composition. The relatively small effect of dietary fatty acids could be due to restricted incorporation of dietary fatty acids into plasma lipids, to the influence of endogenous lipoprotein metabolism on the fatty acid composition of the plasma lipids and to the actions of elongase and desaturase enzymes present in the liver.

The diets used in the present study contained negligible amounts of fatty acids with chain lengths longer than 18 carbons. Hence factors other than dietary intake were involved in determining the amounts of these longer chain PUFA in plasma PLs and CEs. A major factor in determining the amounts of these longer chain PUFA in the PLs and CEs could be the rate of elongation and desaturation of the parent n-3 and n-6 PUFA.

The CAN and S/O/F diets, which were rich in LNA, were associated with significantly higher levels of plasma LNA,

EPA, DPA and pooled n-3 PUFA. The EPA level, in particular, was higher. The level in the PL fractions and in CEs following the CAN and S/O/F diets was more than double the level on the other two experimental diets. These differences suggest that LNA, the parent compound of the n-3 PUFA series, can be elongated and desaturated to longer chain n-3 PUFA in the human body. This conversion has also been demonstrated in other metabolic studies (Mest et al, 1983; Sanders and Roshanai, 1983; Budowski et al, 1984; Lasserre et al, 1985; Adam et al, 1986; Renaud et al, 1986). The in vivo conversion of dietary LNA to EPA, however, is not as efficient in raising the EPA level in PLs as direct EPA supplementation, which has been shown to induce up to a four-fold increase in plasma or platelet EPA levels (Sanders and Roshanai, 1983; Sanders and Younger, 1981).

The difference in the n-3 PUFA levels in plasma lipids following the CAN and SOY diets, which had the same LNA content, suggests that the in vivo synthesis of long chain n-3 PUFA depends on more than just the availability of the precursor, namely LNA, in the system. The high LA content of the SOY diet is assumed to be responsible for the lower n-3 PUFA levels found following the SOY diet. According to Dyerberg (1986) and Leaf and Weber (1988), the large amount of LA in the conventional diets may interfere with the formation of EPA from LNA, due to the competition between LA and LNA for desaturase and elongase enzymes. In other

words, the ratio of LA/LNA in the diet may have a critical influence on n-3 PUFA metabolism in the body.

In the present study, the fact that the CAN and S/O/F diets, which had similar dietary LA/LNA ratios, resulted in similar increases in plasma n-3 PUFA suggests the ratio is important in influencing n-3 PUFA metabolism. This effect of LA/LNA ratio also was found in a study by Sanders and Younger (1981) who gave linseed oil supplements to vegans and omnivores. The supplement reduced the LA/LNA ratio from 6:1 to 1:1 in the omnivores and led to a doubling in the level of EPA in plasma PC (1.3% to 2.7%); the same supplement, which reduced the ratio of dietary LA/LNA from 16:1 to 3:1 in the vegans, resulted in a three-fold increase in plasma PC EPA (0.3% to 1.0%). Similar results were reported by Marshall and Johnston (1982), where diets with different LA/LNA ratios were given to groups of rats. It was found that lowering the ratio to 0.3:1 and 1:1 led to significant increases in the n-3 PUFA levels in liver PE. By contrast, low levels of EPA were seen with ratios of 7:1 and 32:1. The increase in n-3 PUFA following the decrease in LA/LNA ratio could be due to the effective competition of LNA over LA for the $\Delta 6$ desaturase enzyme (Ziboh and Chapkin, 1988).

Although the SOY diet had a much lower LA/LNA ratio than the S/O diet, both diets had a negligible effect on the synthesis of longer chain n-3 PUFA. This finding suggests

that the ratio has to be fairly low, for instance less than 7:1 in the present study, to have a significant effect on the n-3 PUFA metabolism.

On the other hand, the effect of the CAN diet on n-6 PUFA metabolism was not as pronounced as the effect of the SOY and S/O/F diets, even though it contained the same amount of LNA as the SOY diet and had a similar LA/LNA ratio as the S/O/F diet. There is no satisfactory explanation for these different outcomes. The lower plasma n-6 PUFA levels found following the S/O/F diet could be due to the inhibitory effect of LNA on the conversion of LA to AA (Ziboh and Chapkin, 1988). Yet the same change was not found with the CAN diet, probably due to its lower LNA content compared to the S/O/F diet.

In a long term diet study by Lasserre et al (1985), it was shown that a diet providing 4.5% of total energy as LA and 1.5% as LNA (slightly less than the percentage of LNA found in the CAN and SOY diets, see appendix 4) had the same effect on serum n-6 PUFA levels as a diet providing 6.5% of total energy as LA and negligible amount of LNA. Results, therefore, indicated that the presence of LNA of up to 1.5% of total energy in the diet had no suppressive effect on serum levels of n-6 PUFA. According to Sanders and Roshanai (1983), supplementing the diets of normal subjects with 20 ml of linseed oil, which was equivalent to 9g of LNA per day (similar to the amounts found in the CAN and SOY diets), did

not lead to significant changes in the platelet AA levels, even though the levels of LNA and EPA were significantly higher and the level of DTA significantly lower following the supplementation. Supplementing subjects with 30 ml linseed oil per day (approximately 16g/day of LNA, which was similar to the amount present in the S/O/F diet), on the other hand, led to significant decreases in the plasma LA and AA levels (Mest et al, 1983). Supplementing the diet with 60 ml of linseed oil for 6 weeks (Budowski et al, 1984), again, was associated with significantly lower levels of AA in the plasma. Results from these studies, as well as the present study, suggest that higher LNA intakes (above 9g/day) are more likely to suppress plasma AA level.

Contrary observation, however, also exists. In a study conducted by Adam et al (1986), plasma AA level was not suppressed even with a diet containing up to 16% of total energy intake as LNA and a LA/LNA ratio of 0.25. However, the experimental period in this study lasted only two weeks. The intervention period may have been too short to have an effect on plasma AA. Shifting from a normal diet to one that was higher in LNA level (1% of total energy, which was about half that present in the CAN and SOY diets, see appendix 4) for a year resulted in significantly lower levels of plasma AA in European farmers (Renaud et al, 1986). Again, whether or not the duration of the dietary change played a role in influencing the results is not

clear.

The lower ETA and pooled n-6 PUFA levels observed following the SOY diet would not appear to be due to the presence of LNA in the diet as the same changes on plasma n-6 PUFA levels were not observed following the CAN diet, which had the same amount of LNA as the SOY diet. Since both the SOY and S/O/F diets were higher in LA content than the CAN diet and both were associated with lower plasma n-6 PUFA levels, it is possible that the LA content of the diet played a role in influencing the n-6 PUFA metabolism in the plasma. Lasserre et al (1985) suggested the possibility of a suppressive effect of high LA intake on plasma AA level. The study involved feeding diets with different fat sources for periods of 5 months duration to 24 female subjects. A diet providing 14% of total energy as LA (similar to the percentage used in the SOY diet, see appendix 4) and negligible amounts of LNA resulted in significantly lower levels of ETA and AA in serum PLs and CEs. Significantly higher serum levels of ETA and AA were found when LA supplied approximately 6.5% of total energy (similar to the percentage used in the S/O and CAN diets, see appendix 4). Lasserre et al (1985) suggested that the suppressive effect of dietary LA on the serum levels of n-6 PUFA might be due to competition of the fatty acids for the 2-acyl position of the PLs. The possibility of a suppressive effect of dietary LA on AA metabolism also has been mentioned by other

investigators (Dupont, 1987; Kinsella, 1986).

Ferretti et al (1985) conducted a study designed to look at the effect of dietary LA on plasma n-6 PUFA levels. Two levels of LA (3% and 8% of total energy) were used. These levels were similar to those present in the mixed fat and the CAN and S/O diets in the present study. LA intake had no effect on plasma AA level. Likewise, in the present study, there were no consistent or significant differences in plasma AA level between the mixed and the S/O and CAN diets. Ferretti et al (1985) suggested the lack of response of plasma AA to dietary manipulation probably is due to the fact that AA levels in plasma are influenced by several biochemical processes. The effect of diet thus becomes negligible. Similarly, an increase in LA intake from 4.9 to 9.1% of total energy in a long term diet study (Renaud et al, 1986) did not result in any significant changes in plasma ETA and AA levels. Results from these studies (Lasserre et al, 1985; Ferretti et al, 1985; Renaud et al, 1986), as well as the present study, suggest that LA intakes less than 9% of total energy may have very little effect whereas LA intakes around 14% of total energy may have a suppressive effect on plasma n-6 PUFA metabolism. Interestingly, results obtained from the correlation analysis of plasma fatty acids in the present study indicated that the LA level was negatively correlated to the levels of ETA, AA and DTA in most PL and CE fractions.

Dietary fat source had no effect on plasma DHA level in the present study. Similar findings were also reported by Lasserre et al (1985), Renaud et al (1986), Sanders and Roshanai (1983) and Sanders and Younger (1981) regarding the effect of dietary LNA on plasma or platelet DHA levels. Contrasting evidence, however, was observed by Mest et al (1983), where an increase in DHA level was found following linseed oil supplementation. Although the low activity of $\Delta 4$ desaturase has been mentioned as an explanation for the absence of change in plasma DHA level (Sanders and Younger, 1981), no satisfactory explanation is available for the conflicting results observed in the study by Mest et al (1983).

Although the differences in plasma fatty acid levels induced by dietary changes frequently were statistically significant in the present study, differences often were small in magnitude. Nevertheless the clinical or physiological significance of these small changes may be important. An increase in plasma EPA to less than 2 % of total plasma fatty acids following linseed oil supplementation was found to be associated with striking decreases in platelet sensitivity to collagen (Budowski et al, 1984). In the study by Renaud et al (1986), modification in SFA, LA and LNA intakes were associated with a significant decrease in plasma AA from 7.2 to 6.4% of total fatty acids and a significant increase in plasma EPA

from 0.6 to 0.7%. These relatively small changes, in turn, were found to be associated with decreased platelet aggregation and prolonged clotting time. Bleeding time, TXB2 and 6-keto-PGF1α(stable metabolites of TXA2 and PGI2, respectively) production were measured following the feeding of the different experimental diets in the present study. Although no differences in bleeding time and TXB2 production were found among the diets, 6-keto-PGF1α production and 6-keto-PGF1α/TXB2 ratio were significantly higher following the S/O/F diet than the S/O and SOY diets (Gerrard, unpublished data).

Results of the present study indicated that: dietary OA, LA and LNA had similar cholesterol-lowering effects, which, in turn, may suggest the sharing of a common mechanism of these fatty acids in influencing plasma cholesterol metabolism; dietary fat source had an appreciable effect on plasma lipid fatty acid composition and that EPA could be desaturated and elongated from dietary LNA in humans; and dietary LA/LNA ratio appeared to have an important effect on n-3 PUFA metabolism, whereas the absolute amounts of LA and LNA present in the diet appeared to be more important in influencing n-6 PUFA metabolism.

5.3. SUMMARY AND CONCLUSIONS

Studies looking at the effects of dietary fatty acids on plasma lipid and lipoprotein levels and platelet function suggest that dietary fatty acids may be able to influence both the development of atherosclerosis and thrombosis, and thus, in turn, the development of CHD. The present study was designed to look at the effect of SFA, OA, LA and LNA on plasma lipid and lipoprotein patterns and the effect of LA and LNA and their ratio on the fatty acid composition of plasma lipids.

The study consisted 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. The eight subjects that participated in the study received the mixed fat diet during the pre- and post-experimental periods. During the experimental periods, subjects were randomly assigned to one of the experimental diets: the S/O, CAN, SOY and S/O/F diets. The diets provided 13% of total energy (approximately 3200 kcal per day) as protein, 53% as CHO and 34% as fat. The diets were identical except for added fat source which accounted for approximately 77% of total dietary fat. The S/O, CAN, SOY and S/O/F diets provided 56, 22 and 1; 54, 20 and 7; 25, 45 and 7; and 30, 36 and 13% of total fatty acids as OA, LA and LNA, respectively, and about half the amount of SFA present in the mixed fat diet.

Twelve-hour fasting blood samples were taken at the beginning and end of each experimental period. They were analyzed for plasma TC, LDL-C, VLDL-C, HDL-C and TG levels, and the fatty acid composition of plasma PC, PE, PPE and CEs.

Mean plasma TC, LDL-C and VLDL-C levels were significantly lower, 18%, 22% and 41%, respectively, following the experimental diets than following the mixed fat diet. The decrease in TC level was due primarily to the decrease in LDL-C level. Dietary fat source had no effect on HDL-C level, whereas the TG level was significantly lower following the S/O and S/O/F diets than the CAN diet.

The experimental diets differed in their effects on plasma PL and CE fatty acid patterns. Diets which contained relatively higher amounts 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 consistently associated with significantly higher levels of plasma OA, LA and LNA, respectively. The SOY and S/O/F diets were frequently associated with significantly lower levels of ETA and pooled n-6 PUFA in the plasma. The S/O/F diets also was frequently associated with significantly lower levels of AA, whereas the CAN diet was frequently associated with significantly lower levels of DTA. Both the CAN and S/O/F diets were consistently associated with significantly higher levels of plasma EPA. They also were frequently associated with significantly

higher levels of plasma DPA and pooled n-3 PUFA. Diet changes, however, had no effect on plasma DHA level. Results indicated that diets containing different amounts of OA, LA and LNA, which together comprised approximately 26% of total energy, resulted in similar decreases in plasma TC, LDL-C and VLDL-C; dietary fat source had an appreciable effect on plasma lipid fatty acid composition and that EPA could be formed from dietary LNA in the human body; dietary LA/LNA ratio appeared to have an important effect on n-3 PUFA metabolism, whereas the absolute amounts of LA and LNA present in the diet appeared to be more important in influencing n-6 PUFA metabolism.

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APPENDICES

Appendix 1. A Copy of the Consent Form

HUMAN NUTRITION RESEARCH PROJECT

DEPARTMENT OF FOODS AND NUTRITION
UNIVERSITY OF MANITOBA

CONSENT FORM

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 three 24-hour urine samples will be collected during the 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 Acids[2]	Oils				
	Sunflower	Olive	Soybean	Canola	Flaxseed
	(Percent of total fat)				
14:0	0.1	Tr	0.1	0.1	0.1
16:0	6.0	9.0	10.5	4.3	5.6
16:1	0.1	0.4	0.1	0.2	0.1
18:0	4.6	2.6	3.5	2.0	4.2
18:1	14.5	75.6	22.5	62.0	24.6
18:2	73.1	10.0	53.8	20.1	16.9
18:3	0.4	0.8	8.4	8.0	48.1
20:0	0.3	0.6	0.4	0.8	0.2
20:1	0.2	0.6	0.3	1.7	0.2
20:2	Tr	Tr	Tr	Tr	Tr
22:0	0.8	0.4	0.4	0.4	0.2
22:1	Tr	Tr	Tr	0.3	Tr

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

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

Fatty Acids[2]	Diets				
	Mixed Fat	S/O	CAN	SOY	S/O/F
	(Percent of total fat)				
14:0	4.5	1.5	1.5	1.5	1.6
16:0	23.8	13.0	9.8	14.5	11.7
16:1	1.8	0.8	0.7	0.6	0.6
18:0	12.4	4.7	3.8	5.2	5.6
18:1	38.5	56.0	54.2	24.8	29.6
18:2	12.0	21.9	19.5	44.9	36.0
18:3	1.0	0.8	6.6	6.5	13.4
20:0	0.3	0.4	0.6	0.3	0.3
20:1	0.5	0.4	1.3	0.2	0.1
22:0	Tr	0.3	0.4	0.3	0.4

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

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

	Diets				
Fatty Acids	Mixed Fat	S/O	CAN	SOY	S/O/F
	(Percent of total energy intake)				
SFA	13.8	6.7	5.4	7.3	6.6
OA	13.0	18.9	18.3	8.4	10.0
LA	4.0	7.4	6.6	15.1	12.1
LNA	0.3	0.3	2.2	2.2	4.5

1 Means of four duplicate samples (two of each menu).

Appendix 5. Recipes

Granola		Yield: 56x40 g servings
1 Kg	rolled oats	
125 g	bran	
675 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, mix 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	
9/10 g	oil/fat mix[1]	
70 g	boiling water	
1 ml	salt	

Place rice, oil, boiling water and salt into individual casseroles. Cover.
Bake at 350 °F for 30 minutes.

....Cont'd

Appendix 5 (Cont'd).

Jellied Fruit	Yield: 1 serving
100 g	jello
100 g	canned fruit

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

Mashed Potatoes	Yield: 1 serving
30 g	potato flakes
50 ml	milk
26/27 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
50 ml	water
5/6 g	oil/fat mix[1]

Combine carrots and water in casserole dish.
Microwave on high for 5 minutes.
Let stand 5 minutes. Drain. Add in oil.

Chili	Yield: 1 serving
60 g	cooked ground beef
21/26 g	oil/fat mix[1]
120 g	canned tomatoes
65 g	kidney beans
3 g	dehydrated onions
1.5 ml	Worchestire sauce
2 ml	chili powder

Put ingredients into individual casseroles. Mix well.
Bake at 350 ^{o}F for 30 minutes.
Or microwave on medium-high for 4-5 minutes.

....Cont'd

Chicken and Vegetables		Yield: 1 serving
60 g	cooked chicken breast, in small pieces	
10/16 g	oil/fat mix[1]	
30 g	celery	
40 g	canned mushrooms	
40 g	frozen peas	
40 g	chicken broth	

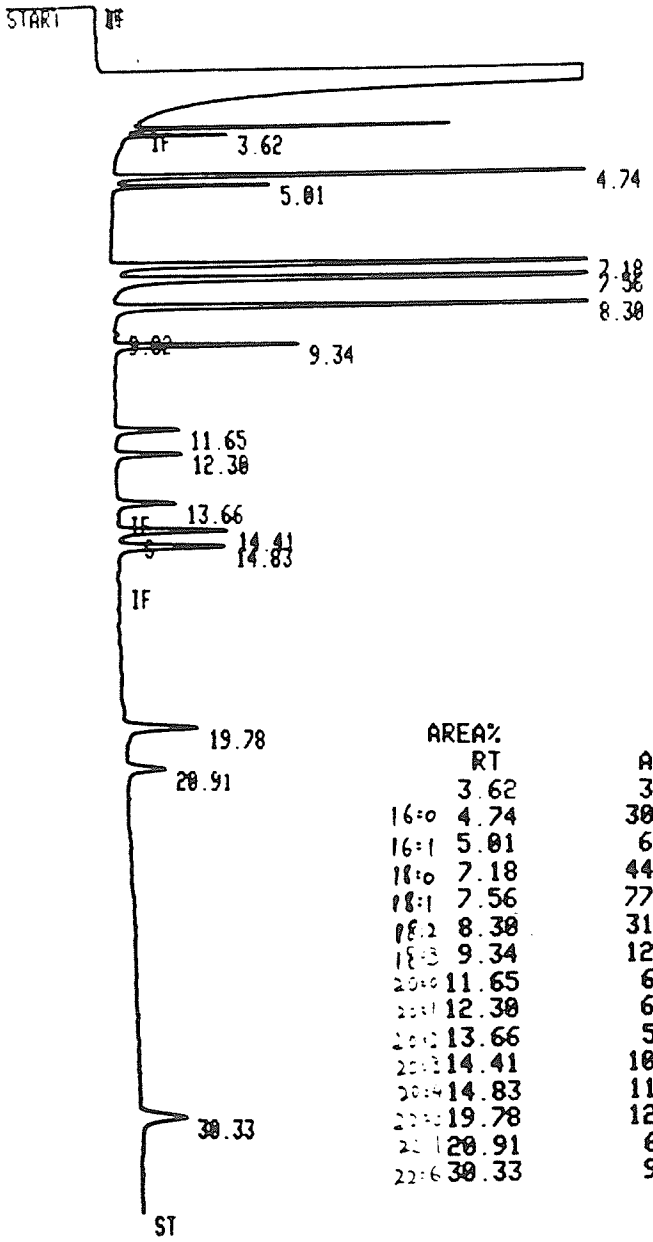
Put ingredients in individual casseroles.
Cover and bake at 350 °F for 30 minutes.
Or microwave on medium-high for 3 minutes.

Noodles		Yield: 1 serving
30 g	noodles	
125 ml	boiling water	
5 ml	oil	
5 g	oil/fat mix[1]	

Place noodles, water and 5 ml oil into individual bowls. Stir and cover.
Microwave on high for 2 1/2 minutes. Let stand for 2 minutes.
Drain. Stir in remaining oil.

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

Appendix 6. Chromatograms of Fatty Acid Standards
A. Chromatogram of the Fatty Acid Standard

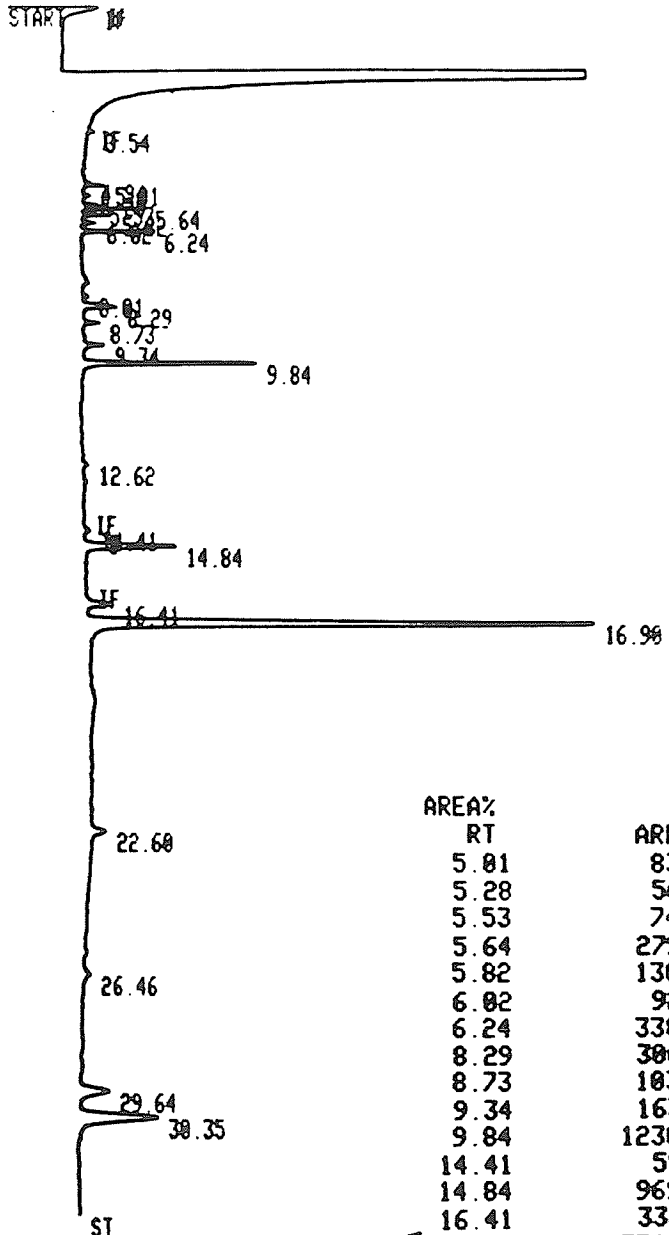


	RT	AREA	TYPE	AR/HT	AREA%
	3.62	3025	BB	0.034	1.094
16:0	4.74	30767	PB	0.042	11.122
16:1	5.01	6073	PB	0.044	2.195
18:0	7.18	44997	PB	0.061	16.266
18:1	7.56	77912	PB	0.063	28.165
18:2	8.30	31946	PB	0.069	11.549
18:3	9.34	12553	PV	0.076	4.538
20:0	11.65	6224	VV	0.105	2.250
20:1	12.30	6035	PV	0.101	2.182
20:2	13.66	5671	BV	0.109	2.050
20:3	14.41	10750	SPB	0.111	3.886
20:4	14.83	11901	PV	0.124	4.302
22:0	19.78	12420	VV	0.184	4.490
22:1	20.91	6505	VV	0.180	2.352
22:6	30.33	9845	PV	0.236	3.559

AREA%

TOTAL AREA= 276620
MUL FACTOR= 1.0000E+00

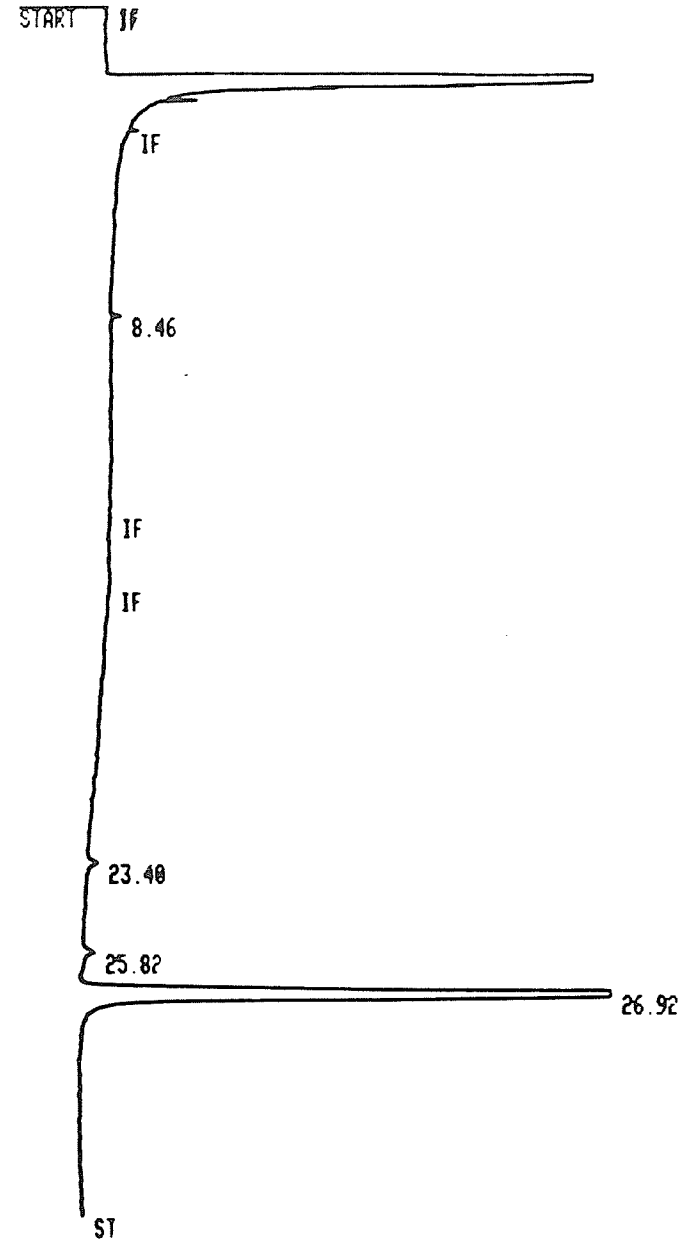
Appendix 6 (Cont'd).
B. Chromatogram of the Eicosapentaenoic Acid (20:5) Standard



	RT	AREA	TYPE	AR/HT	AREA%
	5.01	830	VV	0.044	0.593
	5.28	564	PV	0.078	0.403
	5.53	745	VV	0.051	0.532
	5.64	2790	VV	0.049	1.993
	5.82	1303	VV	0.049	0.931
	6.02	927	VP	0.072	0.662
	6.24	3381	PP	0.052	2.416
	8.29	3064	VV	0.104	2.189
	8.73	1037	VP	0.072	0.741
	9.34	1638	VV	0.089	1.170
	9.84	12305	VV	0.080	8.792
	14.41	598	SPB	0.119	0.427
	14.84	9691	PV	0.120	6.924
	16.41	3327	VV	0.140	2.377
20:5	16.90	73195	PB	0.126	52.295
	22.60	2120	VV	0.171	1.515
	26.46	701	PV	0.126	0.501
	29.64	5866	VP	0.224	4.191
	30.35	15883	PV	0.226	11.348

TOTAL AREA= 139970
MUL FACTOR= 1.0000E+00

Appendix 6 (Cont'd).
C. Chromatogram of the Docosatrienoic Acid (22:3) Standard

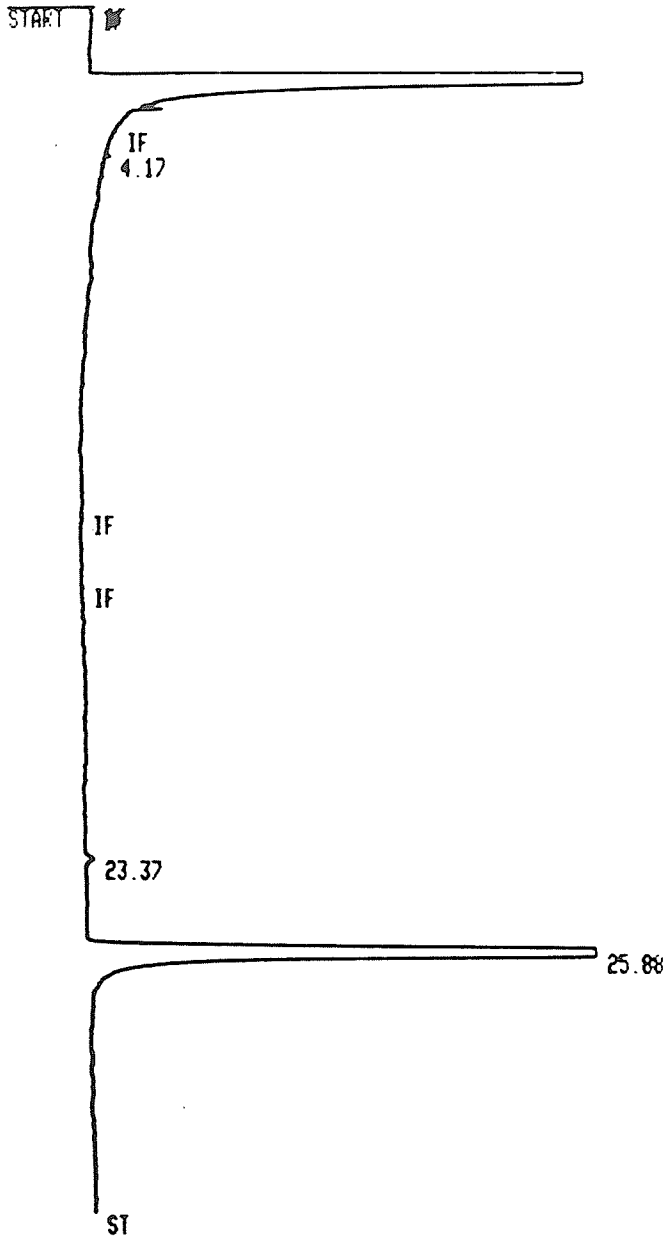


AREA% RT	AREA	TYPE	AR/HT	AREA%
8.46	635	PV	0.067	0.295
23.40	1171	PV	0.138	0.544
25.82	1296	VV	0.142	0.602
22:3 26.92	212150	PB	0.201	98.559

TOTAL AREA= 215260
MUL FACTOR= 1.0000E+00

Appendix 6 (Cont'd).

D. Chromatogram of the Docosatetraenoic Acid (22:4) Standard



	AREA% RT	AREA	TYPE	AR/HT	AREA%
	23.37	1266	BP	0.169	0.372
22:4	25.88	339540	PB	0.211	99.629

TOTAL AREA= 340810
MUL FACTOR= 1.0000E+00

Appendix 7. Formulas for Correction of Fatty Acid Patterns in Phospholipids and Cholesteryl Esters[1]

Formula

eg. An adjustment for palmitic acid.

$$A = \frac{\text{peak area of palmitic acid in the blank}^{2}}{\text{peak area of 15:0}^{3}\text{ in the blank}} \times \text{peak area of 15:0 in sample chromatogram}$$

$$\frac{\text{peak area of palmitic acid in sample chromatogram} - A}{\text{total area of the chromatogram}} \times 100\%$$

= adjusted percentage of palmitic acid

1 Methylation blanks were used for adjusting CE fatty acid values, while TLC blanks were used for adjusting PL fatty acid values.
2 When more than one blank was available, an average ratio of palmitic acid/15:0 obtained from these blanks was used.
3 The internal standard.

Appendix 8. A Sample of Data Analysis

A. Effect Coding of Data

```
INPUT PRETC $ SUB $ DAY $ DIET $ PAM STEAR OA LA LNA ETA
      AA EPA DTA DPA DHA;
      IF PRETC='L' THEN P=-1;
        ELSE IF PRETC='H' THEN P=1;
      IF SUB='S1' THEN SUB1=-1;
        ELSE IF SUB='S6' THEN SUB1=1;
        ELSE SUB1=0;
      IF SUB='S1' THEN SUB2=-1;
        ELSE IF SUB='S2' THEN SUB2=1;
        ELSE SUB2=0;
      IF SUB='S1' THEN SUB3=-1;
        ELSE IF SUB='S5' THEN SUB3=1;
        ELSE SUB3=0;
      IF SUB='S3' THEN SUB4=-1;
        ELSE IF SUB='S8' THEN SUB4=1;
        ELSE SUB4=0;
      IF SUB='S3' THEN SUB5=-1;
        ELSE IF SUB='S9' THEN SUB5=1;
        ELSE SUB5=0;
      IF SUB='S3' THEN SUB6=-1;
        ELSE IF SUB='S4' THEN SUB6=1;
        ELSE SUB6=0;
      IF SUB='S3' THEN SUB7=-1;
        ELSE IF SUB='S7' THEN SUB7=1;
        ELSE SUB7=0;
      IF DAY='D7' THEN DAY1=-1;
        ELSE IF DAY='D25' THEN DAY1=1;
        ELSE DAY1=0;
      IF DAY='D7' THEN DAY2=-1;
        ELSE IF DAY='D43' THEN DAY2=1;
        ELSE DAY2=0;
      IF DAY='D7' THEN DAY3=-1;
        ELSE IF DAY='D207' THEN DAY3=1;
        ELSE DAY3=0;
      IF DAY='D7' THEN DAY4=-1;
        ELSE IF DAY='D225' THEN DAY4=1;
        ELSE DAY4=0;
      IF DAY='D7' THEN DAY5=-1;
         ELSE IF DAY='D243' THEN DAY5=1;
         ELSE DAY5=0;
       IF DIET='SOF' THEN DIET1=-1;
         ELSE IF DIET='CAN' THEN DIET1=1;
         ELSE DIET1=0;
       IF DIET='SOF' THEN DIET2=-1;
         ELSE IF DIET='SOY' THEN DIET2=1;
         ELSE DIET2=0;
       IF DIET='SOF' THEN DIET3=-1;
         ELSE IF DIET='SAO' THEN DIET3=1;
         ELSE DIET3=0;
      Y1=P*DAY1;
      Y2=P*DAY2;
      Y3=P*DAY3;
      Y4=P*DAY4;
      Y5=P*DAY5;
      E1=P*DIET1;
      E2=P*DIET2;
      E3=P*DIET3;
      OMEGAT=EPA+DPA+DHA;
      OMEGAS=ETA+AA+DTA;
```

Appendix 8 (Cont'd).

B. Model Statement and Tests of Differences Between Diet and Day Means

```
PROC REG;
   MODEL LNA = P SUB1--SUB7 DAY1--DAY5 DIET1 DIET2 DIET3
               Y1 Y2 Y3 Y4 Y5 E1 E2 E3/CORR;
     TEST DIET1 - DIET3 = 0;
     TEST DIET1 - DIET2 = 0;
     TEST 2*DIET1 + DIET2 + DIET3 = 0;
     TEST DIET3-DIET2=0;
     TEST 2*DIET3+DIET1+DIET2=0;
     TEST 2*DIET2+DIET1+DIET3=0;
     TEST -2*DAY1 -2*DAY2 - 2*DAY4 - 2*DAY5 = 0;
      TEST 2*DIET1+DAY1+DAY2+DAY4+DAY5=0;
      TEST 2*DIET2+DAY1+DAY2+DAY4+DAY5=0;
      TEST 2*DIET3+DAY1+DAY2+DAY4+DAY5=0;
      TEST -2*DIET1-2*DIET2-2*DIET3+DAY1+DAY2+DAY4+DAY5=0;
```

Appendix 8 (Cont'd).

C. ANOVA Table and Parameter Estimates from Regression Analysis

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
MODEL	24	2.21463313	0.09227638	7.459	0.0001
ERROR	23	0.28453353	0.01237102		
C TOTAL	47	2.49916667			

ROOT MSE	0.1112251	R-SQUARE	0.8861
DEP MEAN	0.3208333	ADJ R-SQ	0.7673
C.V.	34.66756		

PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR HO: PARAMETER=0	PROB > !T!
INTERCEP	1	0.33693204	0.01781087	18.917	0.0001
P	1	-0.01413634	0.01781087	-0.794	0.4355
SUB1	1	-0.06559829	0.04208403	-1.559	0.1327
SUB2	1	0.18655846	0.05973746	3.123	0.0048
SUB3	1	-0.10106838	0.04208403	-2.402	0.0248
SUB4	1	0.01053763	0.04202218	0.251	0.8042
SUB5	1	0.02451613	0.05926023	0.414	0.6829
SUB6	1	-0.03946237	0.04202218	-0.939	0.3574
SUB7	1	-0.02279570	0.04202218	-0.542	0.5927
DAY1	1	-0.01613248	0.03667179	0.440	0.6641
DAY2	1	0.10363248	0.03667179	2.826	0.0096
DAY3	1	-0.13584229	0.03746541	-3.626	0.0014
DAY4	1	-0.04893076	0.03883580	-1.260	0.2203
DAY5	1	0.11351410	0.03883580	2.923	0.0077
DIET1	1	0.00808025	0.03504131	0.231	0.8197
DIET2	1	-0.06691998	0.03504131	-1.910	0.0687
DIET3	1	-0.23203836	0.03683420	-6.300	0.0001
Y1	1	-0.06726085	0.03667179	1.832	0.0799
Y2	1	0.02970085	0.03667179	0.810	0.4263
Y3	1	-0.04695341	0.03746541	-1.253	0.2227
Y4	1	-0.00336246	0.03883580	-0.087	0.9317
Y5	1	-0.02628291	0.03883580	-0.677	0.5053
E1	1	-0.01211228	0.03504131	-0.346	0.7327
E2	1	-0.03711228	0.03504131	-1.059	0.3006
E3	1	-0.01809605	0.03683420	-0.491	0.6279

Appendix 8 (Cont'd).

D. Results of F Tests on Diet and Day Means

TEST: CAN vs S/O	NUMERATOR:	0.204139	DF:	1	F VALUE:	16.5014
	DENOMINATOR:	0.012371	DF:	23	PROB >F :	0.0005
TEST: CAN vs SOY	NUMERATOR:	0.0225	DF:	1	F VALUE:	1.8188
	DENOMINATOR:	0.012371	DF:	23	PROB >F :	0.1906
TEST: CAN vs S&F	NUMERATOR:	0.283158	DF:	1	F VALUE:	22.8888
	DENOMINATOR:	0.012371	DF:	23	PROB >F :	0.0001
TEST: S/O vs SOY	NUMERATOR:	.0965309	DF:	1	F VALUE:	7.8030
	DENOMINATOR:	0.012371	DF:	23	PROB >F :	0.0103
TEST: S/O vs S/O/F	NUMERATOR:	0.937515	DF:	1	F VALUE:	75.7831
	DENOMINATOR:	0.012371	DF:	23	PROB >F :	0.0001
TEST: SOY vs S/O/F	NUMERATOR:	0.453265	DF:	1	F VALUE:	36.6392
	DENOMINATOR:	0.012371	DF:	23	PROB >F :	0.0001
TEST: Mix vs Expt'l	NUMERATOR:	0.458877	DF:	1	F VALUE:	37.0929
	DENOMINATOR:	0.012371	DF:	23	PROB >F :	0.0001
TEST: Mix vs CAN	NUMERATOR:	0.159139	DF:	1	F VALUE:	12.8638
	DENOMINATOR:	0.012371	DF:	23	PROB >F :	0.0016
TEST: Mix vs SOY	NUMERATOR:	.0393511	DF:	1	F VALUE:	3.1809
	DENOMINATOR:	0.012371	DF:	23	PROB >F :	0.0877
TEST: Mix vs S/O	NUMERATOR:	.0530555	DF:	1	F VALUE:	4.2887
	DENOMINATOR:	0.012371	DF:	23	PROB >F :	0.0498
TEST: Mix vs S&F	NUMERATOR:	1.19734	DF:	1	F VALUE:	96.7857
	DENOMINATOR:	0.012371	DF:	23	PROB >F :	0.0001

Appendix 9. Calculation of Adjusted Means and Standard Error

Without adjustment, mean values would be confounded by the presence of a different set of data contributed by subject 9 in the second replicate.

Adjustment:

Adjusted Diet Mean = Intercept + Mean Experimental Day Effect + Diet Effect

Adjusted Day Mean = Intercept + Day Effect

Intercept -- the B value[1] of intercept.
Mean Experimental Day Effect -- mean of the B values of days in which experimental diets were fed.
Diet Effect -- the B value of a specific diet.
Day Effect -- the B value of a specific day.

Standard Error Calculation

Variance of Diet Mean:

= Variance (Intercept + 1/4 Day25 + 1/4 Day43 + 1/4 Day2-25[2] + 1/4 Day2-43[3] + diet Effect)

= Variance (Intercept) + Variance (Diet Effect) + 1/16 Variance (Day 25) + 1/16 Variance (Day 43) + 1/16 Variance (Day 2-25) + 1/16 Variance (Day 2-43)
+ 2 covariance (Intercept.Diet Effect) + 2/4 covariance (Intercept.Day 25) + 2/4 covariance (Intercept.Day 43) + 2/4 covariance (Intercept.Day 2-25) + 2/4 covariance (Intercept.Day 2-43)
+ 2/4 covariance (Diet Effect.Day 25) + 2/4 covariance (Diet Effect.Day 43) + 2/4 covariance (Diet Effect.Day 2-25) + 2/4 covariance (Diet Effect.Day 2-43)
+ 2/16 covariance (Day 25.Day 43) + 2/16 covariance (Day 25.Day 2-25) + 2/16 covariance (Day 25.Day 2-43)
+ 2/16 covariance (Day 43.Day 2-25) + 2/16 covariance (Day 43.Day 2-43)
+ 2/16 covariance (Day 2-25.Day 2-43)

$\sqrt{\text{Variance}}$ = Standard Error

1 The B value refers to the estimated effect of specific parameter as obtained from regression analysis.
2 Day 25 of the second replicate.
3 Day 43 of the second replicate.

Appendix 10. Plasma Lipid and Lipoprotein Levels of Individual Subjects During the Study

Subject	Triglyceride (mmol/L)				
	Replicate I (Day/Diet)				
	D1	D7 Mix	D25 CAN	D43 S/O/F	D49 Mix
1	0.65	0.76	1.13	0.86	1.53
3	0.78	1.23	0.58	0.75	0.95
	D1	D7 Mix	D25 S/O	D43 SOY	D49 Mix
6	0.62	0.76	0.81	0.71	0.87
8	0.71	1.40	0.53	1.11	1.68
	D1	D7 Mix	D25 SOY	D43 CAN	D49 Mix
2	1.01	1.01	0.93	0.97	0.99
4	0.90	1.24	0.58	1.00	0.93
	D1	D7 Mix	D25 S/O/F	D43 S/O	D49 Mix
5	0.48	0.57	0.46	0.36	0.72
7	1.16	0.88	0.73	0.57	0.85
	Replicate II (Day/Diet)				
	D1	D7 Mix	D25 S/O	D43 SOY	D49 Mix
1	1.72	1.02	0.93	0.85	1.35
3	1.33	0.70	0.72	0.64	0.89
	D1	D7 Mix	D25 CAN	D43 S/O/F	D49 Mix
6	0.73	0.92	0.83	0.59	1.03
8	1.58	1.40	1.39	0.94	1.64
	D1	D7 Mix	D25 S/O/F	D43 S/O	D49 Mix
9	1.68	1.28	1.30	1.30	1.24
4	1.45	0.93	0.62	0.60	0.84
	D1	D7 Mix	D25 SOY	D43 CAN	D49 Mix
5	0.62	0.70	0.56	0.68	0.58
7	0.63	0.71	0.58	0.68	0.81

.... Cont'd

Appendix 10 (Cont'd)

Subject	Total Cholesterol (mmol/L)				
Replicate I (Day/Diet)					
	D1	D7 Mix	D25 CAN	D43 S/O/F	D49 Mix
1	4.38	3.98	3.85	4.02	4.68
3	4.71	4.73	3.37	3.66	3.96
	D1	D7 Mix	D25 S/O	D43 SOY	D49 Mix
6	4.61	4.79	4.10	4.14	4.94
8	4.66	4.26	3.38	3.59	4.17
	D1	D7 Mix	D25 SOY	D43 CAN	D49 Mix
2	4.24	3.91	2.88	2.82	3.39
4	5.37	4.77	3.37	3.93	4.34
	D1	D7 Mix	D25 S/O/F	D43 S/O	D49 Mix
5	3.45	3.35	3.24	2.98	3.71
7	5.33	4.64	3.88	3.89	4.55
Replicate II (Day/Diet)					
	D1	D7 Mix	D25 S/O	D43 SOY	D49 Mix
1	5.56	4.94	3.83	4.16	4.50
3	4.16	4.55	3.76	3.56	4.11
	D1	D7 Mix	D25 CAN	D43 S/O/F	D49 Mix
6	4.22	5.10	3.82	3.85	4.39
8	4.55	4.48	3.42	3.22	4.15
	D1	D7 Mix	D25 S/O/F	D43 S/O	D49 Mix
9	5.67	5.29	4.49	4.67	5.17
4	4.55	5.02	3.39	3.19	4.41
	D1	D7 Mix	D25 SOY	D43 CAN	D49 Mix
5	3.68	3.65	3.36	2.99	3.81
7	5.32	4.82	3.81	3.78	4.68

.... Cont'd

Appendix 10 (Cont'd)

Subject	Low Density-Lipoprotein (mmol/L)				
	Replicate I (Day/Diet)				
	D1	D7 Mix	D25 CAN	D43 S/O/F	D49 Mix
1	3.02	2.98	2.47	2.72	3.58
3	2.97	3.14	2.11	2.01	2.61
	D1	D7 Mix	D25 S/O	D43 SOY	D49 Mix
6	3.26	3.44	2.71	2.93	3.29
8	3.13	3.24	2.19	2.26	2.45
	D1	D7 Mix	D25 SOY	D43 CAN	D49 Mix
2	2.30	2.72	1.60	1.53	1.97
4	3.57	3.47	2.11	2.55	3.04
	D1	D7 Mix	D25 S/O/F	D43 S/O	D49 Mix
5	1.93	2.04	1.84	1.70	2.34
7	3.23	3.26	2.45	2.56	3.09
	Replicate II (Day/Diet)				
	D1	D7 Mix	D25 S/O	D43 SOY	D49 Mix
1	3.92	3.44	2.68	3.05	3.24
3	2.54	3.04	2.30	2.37	2.71
	D1	D7 Mix	D25 CAN	D43 S/O/F	D49 Mix
6	2.99	3.59	2.61	2.77	3.09
8	2.77	2.68	1.93	2.05	2.58
	D1	D7 Mix	D25 S/O/F	D43 S/O	D49 Mix
9	3.36	3.47	2.99	3.37	3.52
4	3.32	3.68	2.49	1.98	3.15
	D1	D7 Mix	D25 SOY	D43 CAN	D49 Mix
5	2.14	2.00	2.03	1.66	2.52
7	3.59	3.35	2.66	2.43	3.20

.... Cont'd

Appendix 10 (Cont'd)

Subject	High Density-Lipoprotein (mmol/L)				
	Replicate I (Day/Diet)				
	D1	D7 Mix	D25 CAN	D43 S/O/F	D49 Mix
1	1.19	0.78	0.92	0.95	0.95
3	1.40	1.12	1.31	1.28	1.27
	D1	D7 Mix	D25 S/O	D43 SOY	D49 Mix
6	1.06	0.94	1.14	1.09	1.15
8	1.21	0.84	1.14	1.09	1.03
	D1	D7 Mix	D25 SOY	D43 CAN	D49 Mix
2	1.51	1.03	1.14	1.04	1.18
4	1.42	0.97	1.31	1.13	1.11
	D1	D7 Mix	D25 S/O/F	D43 S/O	D49 Mix
5	1.30	1.08	1.37	1.27	1.35
7	1.58	1.03	1.21	1.24	1.24
	Replicate II (Day/Diet)				
	D1	D7 Mix	D25 S/O	D43 SOY	D49 Mix
1	1.27	1.17	0.95	0.91	0.97
3	1.35	1.36	1.34	1.16	1.33
	D1	D7 Mix	D25 CAN	D43 S/O/F	D49 Mix
6	1.13	1.18	1.14	1.01	1.00
8	1.24	1.24	1.11	0.94	1.07
	D1	D7 Mix	D25 S/O/F	D43 S/O	D49 Mix
9	1.62	1.52	1.15	1.35	1.30
4	1.20	1.17	1.03	1.04	1.19
	D1	D7 Mix	D25 SOY	D43 CAN	D49 Mix
5	1.21	1.27	1.31	1.10	1.27
7	1.51	1.33	1.25	1.16	1.37

.... Cont'd

Appendix 10 (Cont'd)

Subject	Very Low Density-Lipoprotein (mmol/L)				
			Replicate I (Day/Diet)		
	D1	D7 Mix	D25 CAN	D43 S/O/F	D49 Mix
1	0.17	0.22	0.46	0.35	0.15
3	0.34	0.47	0.00	0.37	0.08
	D1	D7 Mix	D25 S/O	D43 SOY	D49 Mix
6	0.29	0.41	0.25	0.12	0.50
8	0.32	0.18	0.05	0.24	0.69
	D1	D7 Mix	D25 SOY	D43 CAN	D49 Mix
2	0.43	0.16	0.14	0.25	0.24
4	0.38	0.33	0.00	0.25	0.19
	D1	D7 Mix	D25 S/O/F	D43 S/O	D49 Mix
5	0.22	0.23	0.03	0.01	0.02
7	0.52	0.35	0.22	0.09	0.22
			Replicate II (Day/Diet)		
	D1	D7 Mix	D25 S/O	D43 SOY	D49 Mix
1	0.37	0.33	0.20	0.20	0.29
3	0.27	0.15	0.12	0.03	0.07
	D1	D7 Mix	D25 CAN	D43 S/O/F	D49 Mix
6	0.10	0.33	0.07	0.07	0.30
8	0.54	0.56	0.38	0.23	0.50
	D1	D7 Mix	D25 S/O/F	D43 S/O	D49 Mix
9	0.69	0.30	0.35	0.00	0.35
4	0.03	0.17	0.00	0.17	0.07
	D1	D7 Mix	D25 SOY	D43 CAN	D49 Mix
5	0.33	0.38	0.02	0.23	0.02
7	0.22	0.14	0.00	0.19	0.11

Appendix 11. Percentage Fatty Acid Composition of Plasma Phosphatidylcholine of Individual Subjects During the Study

Subject	Fatty Acid[1]					
	16:0					
	Replicate--Day/Diet					
	I-7[2] Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	48.0	40.7	44.8	33.3	34.3	39.2
3	30.0	26.0	28.7	28.8	28.6	29.4
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	31.7	27.4	27.3	29.0	25.3	27.9
8	33.0	31.2	31.1	35.2	31.9	32.4
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	43.4	36.4	30.8	-	-	-
9	-	-	-	42.3	32.0	37.0
4	30.2	26.6	26.5	30.9	28.7	29.5
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	33.1	31.2	30.6	35.8	30.9	29.3
7	35.1	30.2	30.6	33.9	34.5	30.0
	16:1					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	0.5	0.5	0.5	0.4	0.4	0.4
3	0.3	0.2	0.2	0.2	0.2	0.2
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.4	0.2	0.1	0.4	0.2	0.2
8	0.6	0.3	0.4	0.6	0.5	0.4
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.3	0.4	0.2	-	-	-
9	-	-	-	0.4	0.2	0.3
4	0.5	0.2	0.4	0.5	0.3	0.3
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.4	0.4	0.2	0.5	0.2	0.3
7	0.3	0.3	0.3	0.3	0.3	0.3

... Cont'd

Appendix 11 (Cont'd).

Subject	18:0					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	13.6	12.9	15.5	12.7	12.8	14.9
3	17.2	16.1	18.0	17.1	15.3	16.6
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	15.8	14.8	14.8	14.6	14.3	17.2
8	16.7	17.1	17.8	17.1	17.7	19.3
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	14.8	16.5	16.3	-	-	-
9	-	-	-	14.4	18.5	14.8
4	16.7	16.7	15.5	16.0	16.0	13.7
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	15.6	15.8	14.1	12.9	15.0	14.7
7	18.7	20.4	17.4	18.6	19.0	18.1
	18:1					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	12.4	14.5	8.8	12.8	14.3	8.1
3	13.4	17.3	10.5	12.5	16.8	8.7
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	13.2	15.1	12.6	15.8	15.8	9.5
8	13.7	15.5	9.8	16.2	15.9	10.4
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	14.0	9.5	14.9	-	-	-
9	-	-	-	12.1	9.6	14.0
4	13.2	8.4	15.7	12.2	10.4	15.1
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	13.1	10.2	14.6	13.0	8.7	15.1
7	13.2	10.5	16.6	13.8	9.6	17.6

... Cont'd

Appendix 11 (Cont'd).

Subject	18:2					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	15.9	18.1	21.7	22.9	22.2	24.4
3	27.2	28.5	33.4	28.4	28.6	36.5
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	22.7	23.3	30.5	23.7	25.0	28.6
8	19.4	19.7	25.3	17.4	18.4	25.0
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	17.9	24.1	22.2	-	-	-
9	-	-	-	18.5	24.8	21.6
4	24.7	31.5	24.2	25.7	30.5	27.5
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	24.1	29.0	26.2	22.1	31.2	23.0
7	18.8	23.5	20.3	18.3	22.5	17.4

	18:3					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	Tr	0.2	0.5	0.1	0.1	0.3
3	0.1	0.6	0.8	0.2	0.1	0.3
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.2	0.1	0.6	0.4	0.5	0.7
8	0.2	0.2	0.4	Tr	0.3	0.9
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.6	0.4	0.5	-	-	-
9	-	-	-	0.2	0.7	0.2
4	0.1	0.2	0.4	0.1	0.8	0.1
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.1	0.5	0.1	0.1	0.2	0.5
7	0.2	0.6	0.2	0.2	0.3	0.3

... Cont'd

Appendix 11 (Cont'd).

Subject	20:0					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	0.0	0.2	0.0	0.1	0.1	0.1
3	0.1	0.2	0.1	0.1	0.1	0.1
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.0	0.1	0.1	0.1	0.2	0.1
8	0.2	0.3	0.2	0.1	0.3	0.2
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.3	0.2	0.2	-	-	-
9	-	-	-	0.0	0.1	0.2
4	0.1	0.1	0.1	0.0	0.1	0.1
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.1	0.1	0.1	0.1	0.1	0.2
7	0.2	0.2	0.2	0.2	0.2	0.3
	20:1					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	0.0	0.5	0.0	0.2	0.2	0.1
3	0.2	0.7	0.2	0.2	0.3	0.2
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.2	0.3	0.2	0.6	0.6	0.2
8	0.3	0.0	0.2	0.3	0.5	0.2
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.3	0.2	0.6	-	-	-
9	-	-	-	0.2	0.2	0.3
4	0.2	0.2	0.6	0.1	0.2	0.2
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.2	0.3	0.4	0.1	0.2	0.7
7	0.8	0.3	0.3	0.2	0.2	0.8

... Cont'd

Appendix 11 (Cont'd).

Subject	20:2					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	0.2	0.5	0.2	0.3	0.3	0.4
3	0.5	0.4	0.4	0.3	0.3	0.6
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.4	0.4	0.5	0.4	0.4	0.4
8	0.3	0.3	0.4	0.3	0.4	0.4
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.3	0.4	0.5	-	-	-
9	-	-	-	0.2	0.5	0.3
4	0.4	0.6	0.5	0.2	0.3	0.3
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.4	0.5	0.4	0.3	0.5	0.5
7	0.3	0.6	0.3	0.3	0.5	0.5
	20:3					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	2.2	2.0	1.2	3.5	3.1	1.9
3	1.9	1.3	1.0	1.8	1.3	0.9
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	2.6	3.6	1.6	3.0	2.3	2.1
8	2.6	2.5	1.8	2.2	2.3	1.8
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	1.4	1.7	2.3	-	-	-
9	-	-	-	1.3	1.6	2.0
4	3.5	2.4	2.8	3.4	1.6	2.4
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	2.4	1.6	2.5	2.2	1.6	2.6
7	2.3	1.8	2.0	2.1	1.5	2.0

... Cont'd

Appendix 11 (Cont'd).

Subject	20:4					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	6.1	7.0	5.0	9.5	8.2	7.1
3	5.5	4.9	3.7	5.8	4.5	3.8
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	8.2	11.4	9.0	8.6	10.4	8.9
8	9.0	8.4	10.0	7.3	7.9	6.2
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	4.7	7.4	7.2	-	-	-
9	-	-	-	6.2	7.3	6.9
4	7.7	9.9	9.1	7.8	7.8	8.1
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	6.3	6.5	7.3	7.9	7.3	7.9
7	7.9	8.3	9.3	8.9	8.7	8.6
	20:5					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	0.2	0.5	0.2	0.4	0.2	0.3
3	0.1	0.2	0.1	0.2	0.1	0.1
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.5	0.2	0.4	0.5	1.3	1.1
8	0.6	0.3	0.4	0.6	1.3	1.1
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.3	0.4	0.9	-	-	-
9	-	-	-	0.4	0.7	0.2
4	0.5	0.5	1.2	0.5	0.7	0.3
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.5	0.6	0.2	0.4	0.4	1.0
7	0.5	1.0	0.3	0.5	0.3	1.2

... Cont'd

Appendix 11 (Cont'd).

Subject — 22:4

Replicate--Day/Diet

	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	Tr	Tr	Tr	0.2	0.2	0.05
3	0.2	0.2	0.1	0.2	0.2	0.1

	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.2	0.3	0.2	0.3	0.2	0.1
8	0.3	0.3	0.2	0.3	0.1	0.04

	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	Tr	Tr	0.1	-	-	-
9	-	-	-	Tr	0.2	0.2
4	0.2	0.1	0.1	0.2	0.1	0.2

	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.2	0.04	0.2	0.3	0.1	0.1
7	0.03	Tr	0.2	0.2	0.2	0.2

22:5

	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	Tr	0.5	0.2	0.8	0.4	0.4
3	0.5	0.4	0.4	0.8	0.5	0.3

	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.8	0.5	0.6	0.6	0.9	0.8
8	0.7	0.8	0.7	0.6	0.9	0.4

	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.3	0.4	0.6	-	-	-
9	-	-	-	1.1	0.9	0.5
4	0.5	0.6	0.8	0.6	0.6	0.5

	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.5	0.6	0.5	0.5	0.5	0.8
7	0.3	0.7	0.5	0.7	0.5	0.8

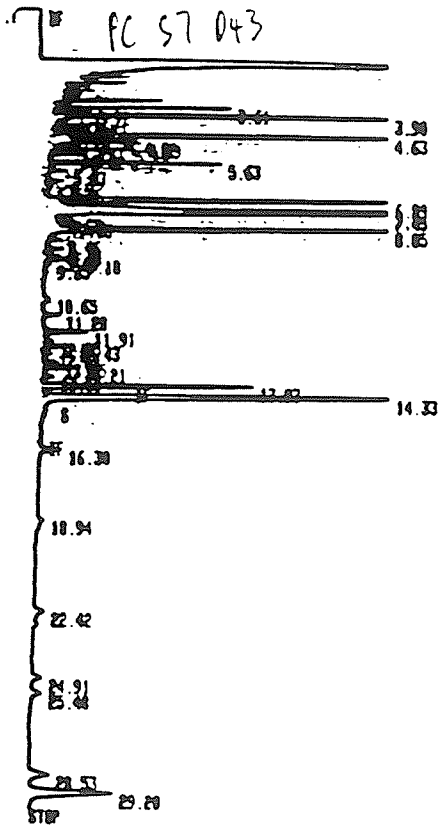
... Cont'd

Appendix 11 (Cont'd).

Subject	22:6					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	1.0	2.0	1.4	2.8	2.9	2.3
3	2.8	2.9	2.3	3.3	3.0	2.1
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	3.1	2.3	1.6	2.1	2.5	2.1
8	2.5	3.1	1.6	1.6	2.0	1.1
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	1.7	2.1	2.8	-	-	-
9	-	-	-	2.7	2.5	1.8
4	1.6	2.0	2.1	1.8	1.8	1.6
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	3.0	2.6	2.5	3.8	3.1	3.2
7	1.5	1.8	1.7	2.0	1.7	2.0

1 Carbon chain length:number of double bond.
2 Replicate I, Day 7, Mixed Fat diet.

Appendix 12. A Sample Fatty Acid Chromatogram of Plasma Phosphatidylcholine (Subject 7, Replicate I, Day 43)



AREA% RT	AREA	TYPE	AREA/WT	AREA%
3.61	6668	VV	0.031	0.320
3.72	2186	VP	0.050	0.105
3.98	660160	PB	0.032	31.659
4.06	335	BP	0.031	0.016
4.16	283	PP	0.044	0.014
4.27	1643	PP	0.044	0.079
4.37	881	PV	0.034	0.038
4.44	563	VV	0.042	0.027
4.63	418520	PB	0.036	20.071
4.74	527	BP	0.034	0.025
4.82	2786	PV	0.035	0.134
4.89	3395	VP	0.037	0.163
4.99	614	PV	0.033	0.030
5.13	5400	PP	0.059	0.259
5.32	1991	PP	0.047	0.096
5.63	10098	PV	0.047	0.484
5.93	1584	VV	0.072	0.076
6.32	848	VP	0.046	0.041
6.52	177	PB	0.060	0.009
6.99	237800	PB	0.051	11.404
7.34	197590	BV	0.056	9.476
7.45	28554	VP	0.060	1.369
7.63	218	PP	0.043	0.010
7.72	567	PP	0.052	0.027
7.83	884	PV	0.052	0.042
8.05	277800	PB	0.058	13.322
8.46	1833	BP	0.066	0.050
8.81	1994	VP	0.087	0.096
9.04	1455	PV	0.078	0.070
9.18	3258	VV	0.074	0.156
9.35	604	VV	0.082	0.029
10.65	965	VV	0.121	0.046
11.28	2137	PP	0.101	0.103
11.91	4839	VP	0.093	0.232
12.25	871	VV	0.089	0.042
12.43	2822	VP	0.088	0.145
12.69	1884	PP	0.091	0.048
13.05	1984	PV	0.107	0.095
13.21	4885	VV	0.113	0.234
13.58	1569	VP	0.108	0.075
13.93	26824	PB	0.103	1.286
14.33	126850	SBB	0.102	6.083
16.38	3126	VP	0.118	0.150
18.94	685	VV	0.069	0.029
22.42	2866	PV	0.211	0.137
24.91	2371	VV	0.186	0.114
25.48	2478	PV	0.193	0.119
28.53	5766	VV	0.223	0.277
29.20	22737	VV	0.221	1.090

TOTAL AREA= 2085200
MUL FACTOR= 1.0000E+00

65.6

Appendix 13. Levels of Significance for Mean Fatty Acid Levels in Plasma Phosphytidylcholine Following Each of the Experimental Diets Compared to those Following the Mixed Fat Diet.

	Experimental Fat Diets			
Fatty Acid(s)[1]	S/O	CAN	SOY	S/O/F
16:0	0.02	0.005	0.21	0.23
18:0	0.03	0.54	0.08	0.0001
18:1	0.0001	0.0001	0.0001	0.0001
18:2	0.29	0.11	0.0001	0.0001
18:3	0.05	0.002	0.09	0.0001
20:3	0.06	0.48	0.0002	0.0001
20:4	0.05	0.22	0.19	0.03
20:5	0.002	0.0001	0.05	0.0009
22:4	0.07	0.19	0.13	0.002
22:5	0.45	0.03	0.39	0.70
22:6	0.50	0.50	0.18	0.09
20:3+20:4+22:4	0.02	0.53	0.59	0.001
20:5+22:5+22:6	0.61	0.007	0.10	0.65

1 Carbon chain length:number of double bond.

Appendix 14. Percentage Fatty Acid Composition of Plasma Phosphatidylethanolamine of Individual Subjects During the Study

Subject	Fatty Acid[1] 16:0					
	Replicate--Day/Diet					
	I-7[2] Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	20.8	13.7	12.0	17.1	12.2	13.2
3	12.8	11.3	11.2	14.3	15.6	11.0
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	13.4	9.3	10.6	12.8	8.7	11.1
8	13.5	11.4	11.0	15.1	10.6	8.8
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	9.9	9.2	9.5	-	-	-
9	-	-	-	8.7	10.3	9.5
4	11.9	9.7	6.5	11.1	9.1	10.2
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	16.0	14.6	15.1	19.7	16.6	14.2
7	11.6	9.6	9.1	16.3	11.2	9.5
	16:1					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	0.7	0.5	0.5	0.5	0.3	0.4
3	0.0	0.0	0.0	0.0	0.1	0.0
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.2	0.2	0.2	0.2	0.1	0.2
8	0.6	0.3	0.2	0.4	0.2	0.2
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.3	0.3	0.2	-	-	-
9	-	-	-	0.5	0.2	0.3
4	0.1	0.1	0.0	0.0	0.0	0.0
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.3	0.4	0.4	0.4	0.2	0.4
7	0.2	0.4	0.2	0.4	0.3	0.4

... Cont'd

Appendix 14 (Cont'd).

Subject	18:0					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	29.0	27.1	32.0	34.7	27.0	30.9
3	30.0	30.3	38.1	32.2	27.3	32.8
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	30.7	26.9	29.0	29.0	24.9	33.1
8	31.6	33.5	33.8	38.6	29.8	33.3
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	31.1	34.0	28.4	-	-	-
9	-	-	-	34.0	32.6	30.1
4	32.1	32.2	34.2	36.1	33.8	29.4
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	37.3	36.6	30.2	30.4	32.5	29.7
7	30.7	31.5	28.1	35.1	30.8	26.9
	18:1					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	13.2	18.4	12.9	14.2	19.5	10.2
3	13.3	19.0	16.6	16.2	16.0	10.9
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	14.2	20.8	18.8	16.6	20.9	14.9
8	13.5	20.6	13.3	14.2	26.1	13.2
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	13.5	9.3	18.4	-	-	-
9	-	-	-	10.8	10.5	14.2
4	14.9	11.6	16.7	13.8	13.3	17.4
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	12.0	13.1	18.9	12.1	11.4	14.6
7	13.3	12.6	19.2	13.8	11.8	17.3

... Cont'd

Appendix 14 (Cont'd).

Subject	18:2					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	12.7	15.4	16.0	11.3	12.0	16.0
3	14.8	16.7	18.9	15.5	17.9	22.8
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-4 S/O/F
6	9.6	9.9	17.6	13.1	12.5	13.5
8	9.7	10.4	16.1	8.7	12.2	17.9
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	13.9	12.2	13.2	-	-	-
9	-	-	-	8.0	15.4	12.7
4	15.8	18.0	4.3	12.4	14.1	12.7
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	9.4	13.8	13.5	11.2	15.0	11.4
7	10.0	13.5	10.9	9.5	13.0	9.2
	18:3					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	0.2	0.5	0.9	Tr	0.6	0.4
3	0.5	0.8	1.3	0.5	0.3	0.6
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.4	0.5	1.0	0.5	0.9	0.9
8	0.1	0.3	0.5	0.1	1.0	1.2
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.1	0.3	0.7	-	-	-
9	-	-	-	Tr	0.8	0.1
4	0.3	0.4	0.4	0.1	0.9	0.1
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.1	0.5	0.1	0.3	0.2	0.5
7	0.1	0.9	0.1	0.1	0.6	0.5

... Cont'd

Appendix 14 (Cont'd).

Subject	20:0					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	0.2	0.3	0.3	Tr	0.4	0.4
3	0.3	0.8	0.5	0.3	0.8	0.5
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.8	0.7	0.8	0.7	0.9	0.7
8	0.2	0.4	0.4	0.2	0.6	0.4
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.3	0.4	0.5	-	-	-
9	-	-	-	0.2	0.2	0.3
4	0.3	0.4	0.9	0.2	0.5	0.2
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.4	0.4	0.4	0.3	0.4	0.3
7	0.2	0.2	0.4	0.2	0.3	0.4
	20:1					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	0.2	0.7	0.4	0.5	0.9	0.4
3	0.5	1.1	0.6	0.4	0.7	0.5
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	2.0	1.4	1.6	1.2	2.0	1.2
8	1.1	0.5	0.4	1.2	0.8	0.4
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.4	0.4	0.9	-	-	-
9	-	-	-	0.5	0.4	0.5
4	0.5	0.4	1.3	0.4	0.5	0.9
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.4	0.4	0.6	0.3	0.4	1.3
7	0.4	0.5	0.6	0.4	Tr	1.0

... Cont'd

Appendix 14 (Cont'd).

Subject	20:2					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	Tr	0.2	0.3	Tr	0.2	0.7
3	0.1	0.8	0.4	0.1	0.3	0.5
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.2	0.4	0.4	0.2	0.7	0.4
8	0.1	0.1	0.4	0.1	0.2	0.4
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.3	0.5	0.3	-	-	-
9	-	-	-	0.5	0.4	0.3
4	0.3	0.5	Tr	0.2	0.3	0.2
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.1	0.4	0.3	0.1	0.4	0.4
7	0.1	0.5	0.4	0.1	0.6	0.4
	20:3					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	1.7	1.3	1.1	1.3	1.4	1.3
3	1.1	1.1	0.6	0.9	0.9	0.7
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	1.2	1.4	0.8	1.4	1.3	0.9
8	1.2	1.2	0.9	1.1	1.1	1.1
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	1.3	1.3	1.4	-	-	-
9	-	-	-	1.5	1.0	1.5
4	1.6	1.2	1.1	1.5	0.7	1.3
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	1.2	0.9	1.3	1.0	0.8	1.4
7	1.6	1.3	1.4	1.1	1.1	1.6

... Cont'd

Appendix 14 (Cont'd).

Subject	20:4					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	16.0	16.3	16.6	17.7	17.0	18.4
3	17.2	13.2	9.0	14.0	14.2	13.3
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	18.3	20.6	14.3	17.5	18.0	15.7
8	20.6	17.1	17.5	15.1	11.7	16.3
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	20.1	23.5	16.8	-	-	-
9	-	-	-	27.7	18.1	22.7
4	17.0	18.5	23.5	18.1	20.0	21.9
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	15.2	13.3	13.9	16.1	15.5	16.9
7	22.9	19.3	21.7	16.7	20.8	21.4
	20:5					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	0.5	0.5	0.6	Tr	0.3	0.4
3	0.3	0.4	0.1	0.3	0.3	0.1
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.4	0.2	0.2	0.4	0.9	0.7
8	0.5	0.3	0.4	0.8	0.8	1.2
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.7	0.5	1.0	-	-	-
9	-	-	-	0.5	0.8	0.4
4	0.5	0.4	1.1	0.5	0.6	0.4
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.4	0.5	0.3	0.3	0.2	1.0
7	0.7	1.4	0.4	0.5	0.6	1.6

... Cont'd

Appendix 14 (Cont'd).

Subject	22:4					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	Tr	0.2	0.4	Tr	0.6	0.4
3	0.8	Tr	0.3	0.5	Tr	0.5
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.6	0.7	0.4	0.7	0.2	0.4
8	0.9	0.4	0.5	0.5	0.2	0.4
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.7	0.5	0.5	-	-	-
9	-	-	-	1.2	0.6	0.8
4	0.5	0.6	0.7	0.7	0.6	0.6
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.5	0.3	0.3	0.5	0.4	0.1
7	0.6	0.4	0.7	0.5	0.6	0.4
	22:5					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	1.0	0.3	1.2	Tr	0.9	1.1
3	1.1	Tr	0.3	0.9	0.9	1.0
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	1.2	0.9	0.8	1.1	1.8	1.6
8	1.5	0.7	1.3	1.0	1.5	1.7
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	1.2	1.2	1.3	-	-	-
9	-	-	-	1.2	2.2	1.5
4	1.0	1.4	2.0	1.3	1.5	1.0
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.8	0.9	0.6	0.8	0.8	1.1
7	1.6	2.1	1.3	1.2	2.0	2.4

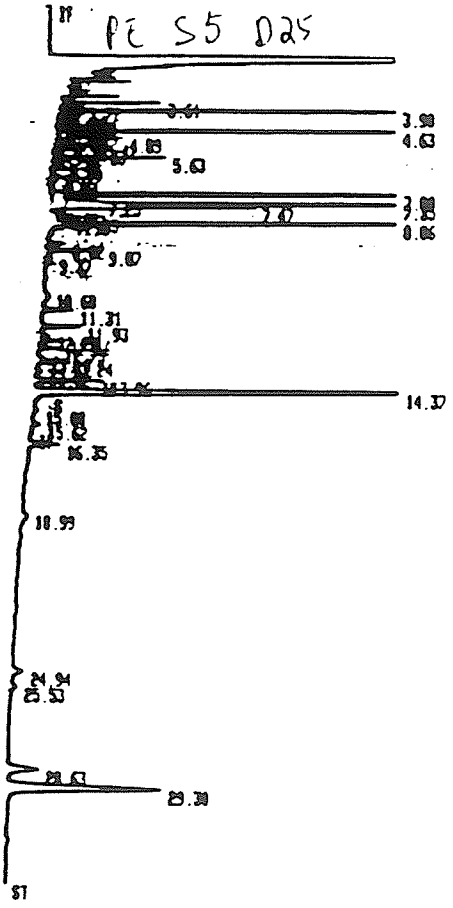
... Cont'd

Appendix 14 (Cont'd).

Subject	22:6					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	3.8	4.7	4.8	2.8	6.9	5.8
3	7.1	4.5	2.0	3.9	4.6	4.7
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	5.9	5.5	2.7	4.0	5.3	4.3
8	5.0	2.9	3.5	3.0	3.0	3.7
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	6.3	6.6	6.8	-	-	-
9	-	-	-	4.8	6.4	5.3
4	3.1	4.8	7.3	3.5	4.0	3.6
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	5.8	4.2	4.4	6.7	5.2	6.6
7	6.0	6.0	5.5	4.1	6.6	7.3

1 Carbon chain length:number of double bond.
2 Replicate I, Day 7, Mixed Fat diet.

Appendix 15. A Sample Fatty Acid Chromatogram of Plasma Phosphatidylethanolamine (Subject 5, Replicate I, Day 25)



AREA% RT	AREA	TYPE	AR/WT	AREA%
3.61	2833	VV	0.032	0.325
3.72	1328	VP	0.054	0.113
3.98	142778	PB	0.033	12.093
4.06	172	BP	0.031	0.015
4.14	921	PV	0.049	0.078
4.27	836	VP	0.059	0.071
4.37	610	PV	0.050	0.052
4.44	722	VP	0.043	0.061
4.55	108	PP	0.037	0.009
4.63	159858	PB	0.038	13.539
4.82	628	BV	0.040	0.053
4.89	3238	VV	0.044	0.274
4.99	336 D	VP	0.035	0.029
5.12	2994	PV	0.059	0.254
5.28	519 D	VV	0.038	0.044
5.33	2038	VP	0.053	0.173
5.56	192	VV	0.046	0.016
5.63	6686	VV	0.047	0.568
5.85	947	VV	0.073	0.080
5.99	1133	VV	0.080	0.096
6.33	527	VP	0.053	0.045
6.53	685	VP	0.096	0.058
7.00	356348	PB	0.051	30.182
7.25	2368	PV	0.058	0.200
7.35	118988	VV	0.057	10.071
7.47	14438	VP	0.059	1.222
7.65	206	PV	0.042	0.018
7.73	676	VV	0.059	0.057
7.85	1494	VV	0.058	0.127
7.92	848	VV	0.057	0.072
8.06	133428	VB	0.059	11.300
8.82	1739	VP	0.079	0.147
9.07	4697	PV	0.069	0.398
9.37	491	VV	0.071	0.042
10.68	573	PV	0.105	0.049
11.31	3389	BP	0.087	0.288
11.93	4018	BP	0.086	0.340
12.28	1278	PV	0.109	0.108
12.46	2735	VV	0.093	0.232
12.75	2163	VV	0.094	0.183
13.18	615	PV	0.091	0.052
13.24	3443	VP	0.101	0.292
13.62	622	BP	0.107	0.053
13.96	8645	PP	0.103	0.732
14.37	128238	BPB	0.101	10.861
15.08	550	PV	0.104	0.047
15.62	1069	PP	0.109	0.091
16.35	4763	PV	0.123	0.403
18.99	416	VV	0.067	0.035
24.94	2528	PV	0.192	0.214
25.53	1203	PV	0.181	0.102
28.63	8138	PP	0.213	0.689
29.38	39748	PV	0.211	3.367

TOTAL AREA= 1180688
MUL FACTOR= 1.0000E+00

Appendix 16. Levels of Significance for Mean Fatty Acid Levels in Plasma Phosphytidylethanolamine Following Each of the Experimental Diets Compared to those Following the Mixed Fat Diet.

	Experimental Fat Diets			
Fatty Acid(s)[1]	S/O	CAN	SOY	S/O/F
16:0	0.02	0.0005	0.03	0.002
18:0	0.002	0.0005	0.99	0.02
18:1	0.0001	0.0001	0.003	0.23
18:2	0.79	0.47	0.0001	0.003
18:3	0.37	0.0001	0.01	0.0001
20:3	0.25	0.42	0.0008	0.0001
20:4	0.58	0.58	0.96	0.08
20:5	0.08	0.0001	0.04	0.003
22:4	0.93	0.006	0.75	0.27
22:5	0.39	0.15	0.47	0.03
22:6	0.58	0.12	0.90	0.54
20:3+20:4+22:4	0.56	0.48	0.77	0.05
20:5+22:5+22:6	0.99	0.04	0.99	0.68

1 Carbon chain length:number of double bond.

Appendix 17. Percentage Fatty Acid Composition of Plasma Alkenylacyl Phosphatidylethanolamine of Individual Subjects During the Study.

Subject	Fatty Acid[1] 16:0					
	Replicate--Day/Diet					
	I-7[2] Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	3.5	Tr	0.9	0.8	4.8	1.9
3	0.9	Tr	0.4	0.3	3.4	2.1
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.8	Tr	0.2	3.7	2.6	1.7
8	0.4	Tr	0.1	1.3	0.9	0.3
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.1	Tr	0.4	-	-	-
9	-	-	-	0.3	1.8	0.3
4	0.4	Tr	0.7	Tr	Tr	Tr
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.5	Tr	1.7	Tr	0.8	2.7
7	Tr	0.9	Tr	Tr	Tr	Tr
	16:1					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	Tr	Tr	Tr	0.1	Tr	Tr
3	Tr	0.1	Tr	Tr	Tr	Tr
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.2	0.1	0.1	Tr	0.1	0.2
8	0.1	Tr	0.1	0.3	0.1	0.2
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.3	0.1	0.1	-	-	-
9	-	-	-	0.1	0.1	Tr
4	Tr	Tr	Tr	Tr	Tr	Tr
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.3	0.1	0.1	0.1	0.1	0.1
7	0.1	0.1	Tr	0.2	Tr	0.1

... Cont'd

Appendix 17 (Cont'd).

Subject	18:0					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	2.1	0.5	0.3	0.4	0.4	0.4
3	0.6	1.2	0.1	0.4	2.4	1.4
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.6	0.5	0.7	2.1	1.6	0.6
8	0.5	0.3	0.4	1.3	0.8	0.6
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.1	1.2	Tr	-	-	-
9	-	-	-	0.4	0.7	0.7
4	0.7	0.1	2.2	0.1	0.2	Tr
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.5	0.2	1.0	0.4	0.6	1.9
7	0.1	1.0	Tr	Tr	0.6	0.8
	18:1					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	8.9	7.6	4.2	5.0	7.3	3.7
3	5.6	11.2	5.7	4.5	10.5	4.4
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	14.9	14.3	9.2	7.3	11.7	6.4
8	9.9	14.3	10.5	13.5	10.2	9.4
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	7.5	6.2	5.2	-	-	-
9	-	-	-	4.7	3.8	6.1
4	4.9	3.3	7.4	5.2	5.4	7.4
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	7.3	4.3	8.0	4.9	4.1	7.0
7	4.2	3.8	4.0	4.5	3.5	5.7

... Cont'd

Appendix 17 (Cont'd).

Subject	18:2					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	8.9	13.0	16.6	11.5	14.1	15.9
3	19.4	14.0	29.9	15.9	19.2	27.2
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	16.4	13.8	19.9	16.7	13.6	20.2
8	10.9	12.0	11.8	10.3	10.5	14.5
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	19.5	17.2	12.6	-	-	-
9	-	-	-	12.9	16.2	13.6
4	11.1	10.3	11.3	11.4	15.8	14.3
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	18.1	18.1	16.9	10.5	18.5	11.7
7	12.9	15.4	10.7	14.1	18.9	11.0
	18:3					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	Tr	0.5	0.6	0.1	Tr	0.4
3	0.3	1.4	1.4	0.3	0.6	0.8
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.8	0.7	0.9	0.3	0.7	0.9
8	0.4	0.7	0.7	0.6	0.6	1.1
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.1	0.6	0.5	-	-	-
9	-	-	-	0.1	0.8	Tr
4	0.1	0.3	0.4	0.1	0.7	Tr
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.1	0.6	0.1	0.1	0.4	0.4
7	0.1	0.5	0.2	0.2	0.4	0.4

... Cont'd

Appendix 17 (Cont'd).

Subject	20:0					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	Tr	Tr	Tr	Tr	Tr	Tr
3	0.1	0.2	Tr	Tr	0.1	Tr
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.6	1.0	0.7	0.5	0.9	0.6
8	0.1	0.3	0.3	0.3	0.3	0.2
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	Tr	Tr	0.1	-	-	-
9	-	-	-	0.1	0.1	Tr
4	Tr	Tr	0.1	Tr	Tr	Tr
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	Tr	Tr	Tr	Tr	0.1	0.3
7	Tr	0.1	Tr	Tr	Tr	Tr
	20:1					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	Tr	0.3	Tr	0.1	Tr	0.1
3	Tr	0.6	0.3	0.3	0.3	0.2
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	1.3	2.0	1.3	1.3	1.9	1.3
8	0.4	0.7	0.4	0.4	0.5	0.3
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.1	0.1	0.3	-	-	-
9	-	-	-	0.1	0.2	0.3
4	0.2	0.1	0.4	0.2	0.1	0.3
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.1	0.1	0.1	0.1	0.1	0.3
7	0.3	0.1	Tr	0.2	0.1	0.4

... Cont'd

Appendix 17 (Cont'd).

Subject	20:2					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	Tr	Tr	Tr	Tr	Tr	Tr
3	0.2	Tr	0.3	0.3	0.1	0.2
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.2	Tr	0.2	Tr	Tr	Tr
8	Tr	Tr	Tr	Tr	Tr	Tr
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	Tr	0.1	0.1	-	-	-
9	-	-	-	Tr	0.1	Tr
4	Tr	Tr	0.1	Tr	Tr	Tr
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	Tr	Tr	0.1	Tr	0.1	0.1
7	Tr	0.1	Tr	Tr	Tr	0.1
	20:3					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	3.3	1.9	1.9	3.0	2.6	1.8
3	2.6	1.9	1.4	3.8	1.8	1.4
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	2.3	1.7	1.1	2.4	1.6	1.5
8	2.6	1.6	1.2	2.5	1.7	2.0
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	2.7	1.6	1.9	-	-	-
9	-	-	-	2.1	1.6	2.3
4	2.7	1.5	1.8	2.7	1.3	1.4
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	2.8	1.6	2.5	2.6	1.3	2.2
7	2.9	1.5	1.9	2.6	1.3	1.9

... Cont'd

Appendix 17 (Cont'd).

Subject	20:4					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	53.9	50.8	49.8	53.8	46.7	48.8
3	41.1	42.2	35.0	45.9	36.1	33.9
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	38.9	46.4	44.2	44.9	43.4	42.1
8	51.7	52.1	53.0	47.0	49.0	44.5
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	46.2	45.6	48.3	-	-	-
9	-	-	-	51.6	44.8	47.9
4	47.7	50.1	45.0	49.7	49.4	50.0
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	44.3	45.9	45.0	49.8	45.3	43.6
7	55.3	48.6	60.3	52.4	51.5	50.8
	20:5					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	2.5	2.7	2.7	2.5	1.7	1.6
3	1.6	1.7	1.4	2.3	1.3	1.1
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	1.9	1.2	1.5	2.9	3.5	3.0
8	2.5	1.0	1.4	2.2	4.6	4.8
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	2.7	1.9	3.8	-	-	-
9	-	-	-	2.2	3.3	1.6
4	2.5	1.7	3.6	2.7	2.4	1.5
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	2.4	3.2	1.6	2.2	1.7	3.9
7	3.3	3.8	1.8	3.5	2.0	5.0

... Cont'd

Appendix 17 (Cont'd).

Subject	22:4					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	1.9	2.5	2.3	3.2	2.4	2.7
3	3.1	2.3	2.3	2.6	2.6	3.2
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	2.7	2.2	2.6	2.9	1.9	2.4
8	3.4	2.6	3.3	3.4	2.5	3.2
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	3.4	3.7	3.0	-	-	-
9	-	-	-	3.7	2.9	4.5
4	4.5	4.8	3.2	4.1	3.7	4.3
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	2.4	2.0	1.9	3.1	2.8	2.0
7	2.5	2.0	2.2	2.4	2.3	2.1
	22:5					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	4.0	4.2	5.5	5.7	5.7	5.1
3	5.9	4.6	4.9	5.9	4.8	6.3
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	5.3	3.5	4.6	4.4	4.5	6.9
8	6.3	4.0	6.9	7.3	7.8	9.7
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	4.7	5.6	5.8	-	-	-
9	-	-	-	7.3	8.5	7.8
4	8.3	8.7	7.7	8.0	7.7	6.9
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	5.5	5.6	4.2	5.5	5.6	6.0
7	5.8	6.9	5.0	6.9	6.4	7.7

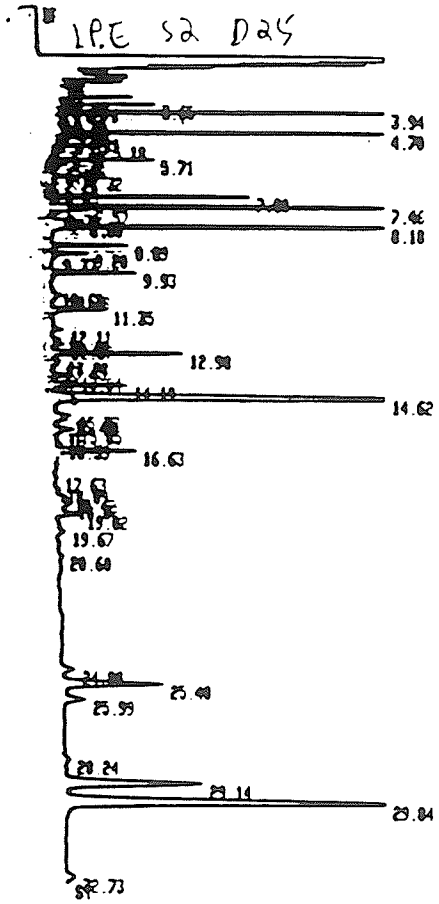
... Cont'd

Appendix 17 (Cont'd).

Subject	22:6					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	11.0	16.1	15.4	13.9	14.5	17.5
3	18.6	18.4	16.8	17.8	16.9	17.8
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	12.4	11.6	12.9	10.7	11.5	11.8
8	10.8	10.4	9.9	9.8	10.6	9.4
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	12.4	16.2	17.9	-	-	-
9	-	-	-	14.3	15.0	14.9
4	16.7	19.1	15.9	15.7	13.1	14.0
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	15.7	18.3	16.9	20.7	18.4	17.9
7	12.6	15.1	14.0	13.1	13.1	14.1

1 Carbon chain length:number of double bond.
2 Replicate I, Day 7, Mixed Fat diet.

Appendix 18. A Sample Fatty Acid Chromatogram of Plasma Alkenylacyl Phosphatidylethanolamine (Subject 2, Replicate I, Day 25)



RT	AREA	TYPE	AREA%	AREA%
3.65	3804	VV	0.033	0.421
3.76	1481	VP	0.064	0.164
3.94	129388	PB	0.034	14.311
4.32	588	VV	0.056	0.056
4.58	764	PV	0.062	0.085
4.70	28565	VB	0.051	3.162
4.89	1141	BV	0.047	0.126
4.95	742	VP	0.058	0.082
5.18	3240	VV	0.054	0.359
5.40	528	PP	0.052	0.058
5.71	5186	PB	0.043	0.574
5.88	696	BP	0.052	0.077
6.32	2048	VV	0.056	0.227
6.60	894	VP	0.117	0.099
6.85	651	PV	0.088	0.072
7.08	13723	BV	0.057	1.519
7.46	45587	VV	0.056	5.037
7.57	2858	VV	0.063	0.316
7.78	727	VP	0.067	0.081
7.96	2151	VV	0.059	0.238
8.03	2138	VV	0.063	0.236
8.18	108348	VB	0.068	11.991
8.89	6591	PV	0.078	0.738
9.20	3397	PV	0.076	0.376
9.37	565	VV	0.083	0.063
9.93	8519	BV	0.081	0.943
10.68	643	PV	0.134	0.071
10.78	1618	VV	0.127	0.179
11.35	5739	PV	0.087	0.635
12.11	1300	VV	0.093	0.144
12.48	1153	PV	0.098	0.128
12.67	1743	VV	0.107	0.193
12.98	15338	VV	0.095	1.698
13.28	719	VV	0.069	0.080
13.45	686	VV	0.092	0.076
13.84	2576	VV	0.101	0.285
14.19	9512	SHB	0.105	1.053
14.62	286820	BB	0.105	31.746
15.33	2232	PV	0.112	0.247
15.49	1765	VP	0.119	0.195
15.85	2811	PV	0.123	0.311
16.39	802	VV	0.109	0.089
16.63	11718	VV	0.119	1.297
18.42	2521	VV	0.174	0.279
18.62	1904	VV	0.105	0.211
19.02	4583	VP	0.103	0.507
19.67	1323	VV	0.147	0.146
24.98	3139	BV	0.192	0.347
25.40	23245	VV	0.109	2.573
25.99	5805	VV	0.207	0.643
28.24	658	PV	0.123	0.072
29.14	35808	PP	0.211	3.963
29.84	101620	BV	0.208	11.248
32.73	2398	PV	0.221	0.265

TOTAL AREA= 903500
AUL FACTOR= 1.0000E+00

69.8 74.7

Appendix 19. Levels of Significance for Mean Fatty Acid Levels in Plasma Alkenylacyl Phosphytidylethanolamine Following Each of the Experimental Diets Compared to those Following the Mixed Fat Diet.

	Experimental Fat Diets			
Fatty Acid(s)[1]	S/O	CAN	SOY	S/O/F
18:1	0.002	0.03	0.02	0.003
18:2	0.95	0.03	0.009	0.0008
18:3	0.44	0.0002	0.002	0.0001
20:3	0.02	0.0002	0.0001	0.0001
20:4	0.89	0.38	0.34	0.04
20:5	0.0001	0.0001	0.0001	0.004
22:4	0.03	0.0001	0.06	0.002
22:5	0.007	0.81	0.53	0.008
22:6	0.68	0.09	0.02	0.97
20:3+20:4+22:4	0.55	0.09	0.12	0.005
20:5+22:5+22:6	0.02	0.01	0.53	0.07

1 Carbon chain length:number of double bond.

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

Subject	Fatty Acid[1] 16:0					
	Replicate--Day/Diet					
	I-7[2] Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	11.4	9.3	8.8	9.8	9.8	12.8[1]
3	8.9	7.7	7.2	8.7	8.5	8.1
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	10.0	10.6	9.6	12.2	10.5	9.0
8	14.3	16.2	10.7	11.9	10.9	8.9
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	8.9	9.9[3]	9.5[3]	-	-	-
9	-	-	-	12.8[3]	9.3[3]	10.3[3]
4	8.9	8.6	7.5	10.2	8.7	10.1
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	8.4	6.9	8.1	7.9	7.7	7.0
7	10.8	10.8	9.9	10.5	9.9	10.8
	16:1					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	6.6	5.2	4.0	6.7	4.6	/
3	3.0	2.0	1.5	2.4	1.3	1.1
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	1.6	0.9	0.7	1.5	0.8	0.6
8	2.3	1.0	1.1	2.1	1.3	1.1
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	4.0	/	/	-	-	-
9	-	-	-	/	/	/
4	3.1	2.7	1.5	3.7	1.0	2.9
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	2.3	1.2	1.4	2.3	1.3	1.1
7	3.5	1.5	1.4	3.0	1.5	1.9

... Cont'd

Appendix 20 (Cont'd).

Subject	18:0					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	1.2	0.4	0.3	0.6	0.2	0.2
3	1.2	0.6	1.3	1.2	0.8	0.7
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.7	0.9	0.7	1.5	0.8	0.8
8	1.5	1.5	0.8	1.3	0.6	0.9
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	Tr	Tr	Tr	-	-	-
9	-	-	-	Tr	0.2	0.2
4	1.0	0.6	0.7	0.7	0.7	0.5
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.8	0.5	0.6	0.9	0.2	0.1
7	1.0	1.0	0.7	1.3	0.6	0.9
	18:1					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	21.1	21.9	14.3	20.7	23.0	13.3
3	20.7	19.9	13.5	18.2	21.9	10.4
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	18.7	21.7	10.6	16.5	17.9	11.0
8	21.7	21.8	13.0	22.4	22.5	13.6
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	21.8	11.8	21.1	-	-	-
9	-	-	-	20.7	14.3	23.9
4	19.6	10.6	20.8	17.6	14.2	20.2
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	20.6	14.6	22.5	20.7	11.8	22.5
7	22.1	12.8	22.6	20.9	11.0	21.8

... Cont'd

Appendix 20 (Cont'd).

Subject	18:2					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	47.1	51.3	61.9	50.5	53.5	64.8
3	59.7	63.6	70.3	62.9	62.8	74.6
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	58.5	54.7	67.3	56.0	56.6	66.4
8	50.1	49.7	63.8	51.2	53.2	63.9
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	56.8	69.3	60.0	-	-	-
9	-	-	-	57.1	66.4	57.2
4	57.0	67.5	57.4	57.9	65.1	58.1
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	59.4	67.1	58.8	58.6	71.0	60.3
7	52.4	62.7	54.2	52.6	65.9	52.9
	18:3					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	0.2	0.6	1.3	0.1	Tr	0.4
3	0.4	1.1	2.1	0.5	0.4	0.8
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.3	0.5	1.0	0.4	1.3	2.4
8	0.4	0.2	1.0	0.5	1.3	2.1
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.1	0.5	0.6	-	-	-
9	-	-	-	0.1	1.9	0.2
4	0.5	0.8	1.2	0.4	1.8	0.2
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.5	1.9	0.4	0.9	0.9	1.3
7	0.2	1.8	0.2	1.0	1.1	1.3

... Cont'd

Appendix 20 (Cont'd).

Subject	20:0					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	Tr	Tr	Tr	Tr	Tr	Tr
3	0.1	0.1	0.2	0.1	0.1	0.1
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	Tr	Tr	0.1	0.1	0.1	0.1
8	0.1	0.5	Tr	Tr	0.1	0.1
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	Tr	Tr	Tr			
9	-	-	-	Tr	Tr	Tr
4	0.1	0.1	0.1	0.1	0.1	0.1
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.1	0.1	Tr	0.1	Tr	Tr
7	Tr	0.1	0.1	0.1	Tr	0.1
	20:1					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	1.8	1.1	0.8	1.0	0.7	0.8
3	Tr	Tr	Tr	Tr	Tr	Tr
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	Tr	Tr	Tr	0.1	0.1	0.1
8	0.1	0.1	Tr	Tr	0.1	0.5
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.5	0.5	0.5	-	-	-
9	-	-	-	0.5	0.6	1.0
4	Tr	Tr	Tr	Tr	Tr	Tr
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.1	0.1	0.3	0.2	0.1	0.1
7	Tr	Tr	0.1	Tr	Tr	Tr

... Cont'd

Appendix 20 (Cont'd).

Subject	20:2					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	Tr	Tr	Tr	Tr	Tr	Tr
3	Tr	Tr	Tr	Tr	Tr	Tr
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	Tr	0.1	0.1	Tr	Tr	0.1
8	Tr	Tr	Tr	Tr	Tr	Tr
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	Tr	Tr	Tr	-	-	-
9	-	-	-	Tr	Tr	Tr
4	Tr	Tr	Tr	Tr	Tr	Tr
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	Tr	Tr	Tr	Tr	Tr	Tr
7	Tr	0.1	0.1	0.3	0.3	0.3
	20:3					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	0.5	0.4	Tr	Tr	Tr	Tr
3	0.4	0.4	0.2	0.5	0.2	0.2
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	Tr	0.8	0.4	0.9	0.8	0.5
8	0.6	0.6	0.5	0.7	0.6	0.6
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	Tr	Tr	Tr	-	-	-
9	-	-	-	Tr	Tr	Tr
4	0.8	0.4	0.7	0.7	0.3	0.3
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.8	1.0	1.1	0.3	0.5	0.8
7	0.9	0.4	0.6	0.5	0.5	0.9

... Cont'd

Appendix 20 (Cont'd).

Subject	20:4					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	8.4	7.6	6.8	9.1	7.7	7.3
3	4.2	3.8	2.7	4.5	3.2	3.1
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	9.7	8.9	8.4	9.2	8.9	7.4
8	8.2	7.8	8.3	8.9	7.6	6.7
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	7.2	7.1	7.6	-	-	-
9	-	-	-	8.7	6.5	6.8
4	7.4	7.4	8.0	7.6	7.0	6.9
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	6.0	5.4	5.6	6.7	5.5	5.7
7	8.5	7.1	9.2	8.7	8.4	7.7
	20:5					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	1.1	1.6	1.6	1.2	0.4	0.2
3	0.7	0.4	0.6	0.4	0.4	0.2
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.1	0.3	0.5	0.6	1.3	1.1
8	0.6	0.2	0.5	0.7	1.4	1.4
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.5	0.6	0.5	-	-	-
9	-	-	-	0.1	0.6	0.1
4	0.9	0.9	1.4	0.6	0.7	0.3
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.5	0.8	0.5	0.5	0.4	0.5
7	0.6	1.1	0.4	0.8	0.5	1.4

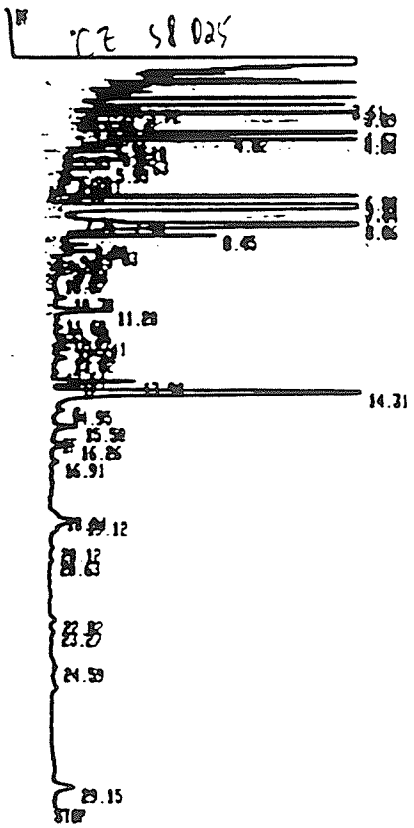
... Cont'd

Appendix 20 (Cont'd).

Subject	22:6					
	Replicate--Day/Diet					
	I-7 Mix	I-25 CAN	I-43 S/O/F	II-7 Mix	II-25 S/O	II-43 SOY
1	0.7	0.6	0.2	0.2	0.2	0.2
3	0.7	0.6	0.5	0.7	0.5	0.7
	I-7 Mix	I-25 S/O	I-43 SOY	II-7 Mix	II-25 CAN	II-43 S/O/F
6	0.4	0.6	0.6	0.9	0.8	0.6
8	0.2	0.3	0.5	0.3	0.4	0.4
	I-7 Mix	I-25 SOY	I-43 CAN	II-7 Mix	II-25 S/O/F	II-43 S/O
2	0.2	0.4	0.2	-	-	-
9	-	-	-	0.1	0.2	0.2
4	0.7	0.5	0.7	0.7	0.5	0.3
	I-7 Mix	I-25 S/O/F	I-43 S/O	II-7 Mix	II-25 SOY	II-43 CAN
5	0.5	0.4	0.8	1.1	0.6	0.7
7	0.1	0.6	0.4	0.4	0.3	0.3

1 Carbon chain length:number of double bond.
2 Replicate I, Day 7, Mixed Fat diet.
3 Values could be the sum of 16:0 and 16:1 due to imperfect separation of the two fatty acids by gas chromatography.

Appendix 21. A Sample Fatty Acid Chromatogram of Plasma Cholesteryl Esters (Subject 8, Replicate I, Day 25)



RELAT RT	AREA	TYPE	AREA HT	AREA%	
3.55	653	PV	0.033	0.026	
3.61	12225	VV	0.038	0.495	
3.72	3898	VP	0.051	0.158	
3.89	148000	PB	0.036	5.994	
4.06	507	BP	0.034	0.021	
4.15	919	PV	0.046	0.037	
4.26	2758	VP	0.056	0.112	
4.37	286	PV	0.032	0.012	
4.44	1253	VP	0.047	0.051	
4.63	374190	PB	0.043	15.145	-16:0
4.82	5665	BV	0.037	0.229	
4.88	21799	VV	0.043	0.882	-16:1
4.98	879	VV	0.038	0.036	
5.10	3949	PP	0.067	0.160	
5.32	2926	PV	0.050	0.118	
5.43	387	VP	0.053	0.016	
5.63	6471	PV	0.079	0.262	
5.83	5115	VV	0.085	0.207	
6.20	914	VV	0.061	0.037	
6.31	1549	VV	0.056	0.063	
6.49	1582	VV	0.104	0.064	
6.74	743	VP	0.071	0.030	
1.3 6.98	40414	PB	0.060	1.636	-18:0
1.4 7.34	471060	BV	0.059	19.068	
7.44	34448	VB	0.067	1.394	-18:1
7.76	315	PP	0.050	0.013	
7.90	1390	PV	0.052	0.056	
8.06	1067200	VB	0.062	43.196	-18:2
8.45	12226	BB	0.066	0.495	
8.88	3427	BV	0.099	0.139	
9.03	3664	VV	0.075	0.148	-18:3
9.15	1499	VP	0.075	0.061	
9.33	326	PV	0.067	0.013	
9.45	986	VP	0.062	0.040	
9.68	1905	PV	0.103	0.077	
9.89	1542	VP	0.096	0.062	
10.07	522	PV	0.108	0.021	
10.78	2138	VV	0.124	0.086	
11.28	9379	VV	0.133	0.380	-20:0
11.68	1048	VV	0.139	0.042	
11.98	1611	VV	0.127	0.065	-20:1
12.22	1700	VV	0.109	0.069	
12.41	3389	VV	0.107	0.137	
12.66	2166	VV	0.101	0.088	
13.02	2503	VV	0.143	0.101	
13.54	791	VV	0.131	0.032	
13.98	11599	BH	0.113	0.470	-20:3
14.31	165850	BB	0.110	6.721	-20:4
14.95	1038	PV	0.110	0.042	
15.58	4339	PV	0.146	0.176	
16.26	3589	VV	0.136	0.146	-20:5
16.91	1487	VP	0.136	0.057	
18.94	1932	PV	0.171	0.078	
19.12	8619	VV	0.235	0.349	
20.12	0	PB	0.000	0.000	
20.63	529	PV	0.115	0.021	
22.82	670	VV	0.095	0.027	
23.27	466	PV	0.108	0.019	
24.59	881	VV	0.120	0.036	
29.15	6386	VV	0.239	0.255	-22:6

TOTAL AREA= 2470700
MUL FACTOR= 1.0000E+00

Appendix 22. Levels of Significance for Mean Fatty Acid Levels in Plasma Cholesteryl Esters Following Each of the Experimental Diets Compared to those Following the Mixed Fat Diet.

	Experimental Fat Diets			
Fatty Acid(s)[1]	S/O	CAN	SOY	S/O/F
16:0	0.58	0.03	0.23	0.005
18:0	0.08	0.004	0.001	0.16
18:1	0.0001	0.0001	0.0001	0.0001
18:2	0.06	0.22	0.0001	0.0001
18:3	0.0003	0.0001	0.008	0.0001
20:3	0.95	0.15	0.12	0.36
20:4	0.01	0.08	0.006	0.0001
20:5	0.004	0.002	0.10	0.003
22:6	0.37	0.52	0.83	0.27
20:3+20:4	0.02	0.35	0.0007	0.0001
20:5+22:6	0.01	0.01	0.21	0.09

1 Carbon chain length:number of double bond.

Appendix 23. Levels of Significance for Diet Means of Fatty Acids in Plasma Phosphatidylcholine

Fatty Acid(s)[1]	Diet Comparison: CAN vs S/O	CAN vs SOY	CAN vs S/O/F	S/O vs SOY	S/O vs S/O/F	SOY vs S/O/F
16:0	0.74	0.19	0.22	0.36	0.38	0.99
18:0	0.21	0.08	0.0002	0.006	0.0001	0.01
18:1	0.20	0.0001	0.0001	0.0001	0.0001	0.29
18:2	0.06	0.0001	0.0001	0.0001	0.0001	0.22
18:3	0.0005	0.19	0.0001	0.01	0.0001	0.0001
20:3	0.06	0.01	0.007	0.0001	0.0001	0.69
20:4	0.51	0.96	0.01	0.54	0.003	0.01
20:5	0.0001	0.0001	0.01	0.29	0.0001	0.0003
22:4	0.03	0.87	0.13	0.02	0.0008	0.17
22:5	0.04	0.02	0.06	0.97	0.78	0.74
22:6	0.98	0.14	0.085	0.15	0.085	0.73
n-6[2]	0.19	0.38	0.004	0.04	0.0002	0.03
n-3[3]	0.02	0.002	0.02	0.43	0.97	0.41

1 Carbon chain length:number of double bond.
2 n-6 = 20:3+20:4+22:4.
3 n-3 = 20:5+22:5+22:6.

Appendix 24. Levels of Significance for Diet Means of Fatty Acids in Plasma Phosphatidylethanolamine

Fatty Acid(s)[1]	Diet Comparison: CAN vs S/O	CAN vs SOY	CAN vs S/O/F	S/O vs SOY	S/O vs S/O/F	SOY vs S/O/F
16:0	0.34	0.23	0.87	0.84	0.44	0.32
18:0	0.84	0.006	0.0001	0.02	0.0002	0.08
18:1	0.71	0.0001	0.0001	0.0001	0.0001	0.16
18:2	0.75	0.0004	0.007	0.002	0.02	0.36
18:3	0.0001	0.045	0.007	0.02	0.0001	0.0001
20:3	0.77	0.002	0.0004	0.002	0.0002	0.41
20:4	0.43	0.71	0.34	0.66	0.09	0.20
20:5	0.0001	0.0001	0.16	0.88	0.001	0.0006
22:4	0.05	0.053	0.21	0.88	0.45	0.54
22:5	0.10	0.59	0.47	0.25	0.03	0.22
22:6	0.48	0.28	0.12	0.75	0.40	0.58
n-6[2]	0.35	0.76	0.29	0.52	0.06	0.18
n-3[3]	0.14	0.12	0.24	0.99	0.77	0.76

1 Carbon chain length:number of double bond.
2 n-6 = 20:3+20:4+22:4.
3 n-3 = 20:5+22:5+22:6.

Appendix 25. Levels of Significance for Diet Means of Fatty Acids in Plasma Alkenylacyl Phosphatidylethanolamine

Fatty Acid(s)[1]	Diet Comparison					
	CAN vs S/O	CAN vs SOY	CAN vs S/O/F	S/O vs SOY	S/O vs S/O/F	SOY vs S/O/F
18:1	0.35	0.001	0.0004	0.0002	0.0001	0.52
18:2	0.11	0.0008	0.0002	0.06	0.01	0.40
18:3	0.001	0.56	0.06	0.005	0.0001	0.02
20:3	0.25	0.009	0.19	0.0008	0.02	0.19
20:4	0.60	0.96	0.31	0.57	0.14	0.34
20:5	0.0001	0.0001	0.11	0.78	0.0001	0.0001
22:4	0.06	0.0001	0.24	0.005	0.45	0.0007
22:5	0.03	0.77	0.06	0.02	0.0003	0.10
22:6	0.13	0.64	0.23	0.06	0.74	0.11
n-6[2]	0.43	0.91	0.30	0.49	0.08	0.26
n-3[3]	0.001	0.14	0.64	0.03	0.004	0.35

1 Carbon chain length:number of double bond.
2 n-6 = 20:3+20:4+22:4.
3 n-3 = 20:5+22:5+22:6.

Appendix 26. Levels of Significance for Dietary Means of Fatty Acids in Plasma Cholesteryl Esters

Fatty Acid(s)[1]	Diet Comparison: CAN vs S/O	CAN vs SOY	CAN vs S/O/F	S/O vs SOY	S/O vs S/O/F	SOY vs S/O/F
16:0	0.049	0.41	0.51	0.21	0.01	0.16
18:0	0.36	0.77	0.24	0.23	0.80	0.15
18:1	0.02	0.0001	0.0001	0.0001	0.0001	0.002
18:2	0.55	0.0001	0.0001	0.0001	0.0001	0.01
18:3	0.0001	0.008	0.0001	0.0001	0.0001	0.0001
20:3	0.32	0.03	0.09	0.25	0.48	0.66
20:4	0.51	0.37	0.0001	0.86	0.0006	0.0009
20:5	0.0001	0.0006	0.92	0.24	0.0001	0.001
22:6	0.27	0.52	0.21	0.60	0.87	0.50
n-6[2]	0.23	0.04	0.0001	0.40	0.0003	0.002
n-3[3]	0.0006	0.005	0.51	0.29	0.004	0.04

1 Carbon chain length:number of double bond.
2 n-6 = 20:3+20:4.
3 n-3 = 20:5+22:6.