EFFECT OF LOW LINOLENIC CANOLA OIL ON PLASMA LIPOPROTEINS AND THE FATTY ACID COMPOSITION OF PLATELET PHOSPHOLIPIDS IN HEALTHY MEN

BY

CARRIE RAE MULLIN

A Thesis
submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

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ABSTRACT

A 61-day study was designed to assess the effects of the level of dietary linolenic acid (LNA) and its ratio to linoleic acid (LA) on plasma lipoproteins (LP) and the fatty acid composition of the platelet phospholipids (PL) in twelve normolipidemic men. The study consisted of four diet periods: a 7-day pre-experimental period and two 18-day experimental periods separated by an 18-day washout period. Diets supplied 53% of total energy as carbohydrate, 14% as protein and 33% as fat. Added fat accounted for 79% of the total fat or 29% of total energy. A mixture of fats (MF) was provided during the pre-experimental and washout periods. Subjects were randomly assigned to receive two of three experimental diets containing: i) 100% low linolenic canola oil (LLNA), ii) 85% regular canola oil and 15% sunflower oil (CAN), or iii) 67% regular canola oil, 15% flax oil, and 18% sunflower oil (FLAX). The experimental diets provided similar amounts of LA but different amounts of LNA. The LA/LNA ratios and the LNA levels of the diets were 6.3, 4.3 and 2.5, and 4%, 6% and 11%, respectively. Fasting 12-hour blood samples were analyzed for plasma lipids and LP. Platelets were isolated from the plasma and the fatty acid composition of the phosphatidylcholine (PC), phosphatidylethanolamine (PE) and alkenylacyl ethanolamine phosphoglyceride (PPE) fractions were determined. Plasma total cholesterol (TC) and low density lipoprotein

cholesterol (LDL-C) decreased on all experimental diets. Plasma triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) decreased ($p<0.05$; $p<0.03$, resp.) on the FLAX diet, whereas very low density lipoprotein cholesterol (VLDL-C) decreased ($p<0.01$) on the CAN diet. Fatty acid analyses indicated that LNA levels of PL increased on the experimental diets. Levels of eicosapentaneoic acid (EPA) increased on the FLAX diet but were unaffected by the LLNA and CAN diets. Levels of long chain (LC) n-3 PUFA decreased on the LLNA diet, were unchanged on the CAN diet, but showed a trend towards increased levels on the FLAX diet. By contrast, platelet AA levels and n-6 LC PUFA levels decreased on all experimental diets. Thus, the LLNA diet (LA/LNA ratio of 6.3) reduced n-3 LC PUFA levels in platelet PL, primarily as a result of a decrease in the DPA level. Diets with lower LA/LNA ratios (2.5 and 4.3) maintained total n-3 LC PUFA levels, however only the diet with the lowest LA/LNA ratio increased platelet EPA levels.

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LIST OF ABBREVIATIONS

Alkenylacyl Ethanolamine Phosphoglyceride	PPE
Arachidonic Acid	AA
Balanced Incomplete Block Design	BIBD
Canola Diet	CAN
Carbohydrate	CHO
Coronary Heart Disease	CHD
Docosahexanenoic Acid	DHA
Docosapentaenoic Acid	DPA
Docosatetraenoic Acid	DTA
Eicosadecaenoic Acid	EDA
Eicosapentaenoic Acid	EPA
Eicosatrienoic Acid	ETA
Flax Diet	FLAX
Gas Chromatography	GC
High Density Lipoprotein-Cholesterol	HDL-C
Linoleic Acid	LA
Linolenic Acid	LNA
Lipoprotein	LP
Low Density Lipoprotein-Cholesterol	LDL-C
Low Linolenic Canola Oil	low-LNA
Monounsaturated Fatty Acids	MUFA
Oleic Acid	OA
Palmitic Acid	PMA
Pentadecaenoic Acid	15:0

ABBREVIATIONS (cont'd).

Phosphatidylcholine	PC
Phosphatidylethanolamine	PE
Phospholipids	PL
Polyunsaturated Fatty Acids	PUFA
Prostacyclin	PGI_2
Saturated Fatty Acids	SFA
Stearic Acid	STEA
Thin-Layer Chromatography	TLC
Thromboxane	TX
Total Cholesterol	TC
Triglyceride	TG
Unsaturated Fatty Acids	unSFA
Very Low Density Lipoprotein-Cholesterol	VLDL-C

1. LITERATURE REVIEW

1.1 Introduction

Dietary fat, both the amount and type, is strongly associated with the development of atherosclerosis and thrombosis, the two main processes of coronary heart disease (CHD) (Ulbricht and Southgate, 1991). The pathological basis of CHD is atherosclerosis which often takes years to develop, whereas thrombosis may develop in only a few hours (Truswell, 1985). In many industrialized nations, 20 - 30% of deaths among males between the ages of 40 - 69 years are attributable to CHD. However, there has been a general decline in mortality due to CHD in the past two decades (Uemura, 1985). This improvement partly reflects the changing dietary habits of North Americans (Nordoy and Goodnight, 1990).

Epidemiological studies have demonstrated a positive relationship between high levels of plasma cholesterol and the occurrence of CHD (Keys et al., 1957; Dyerberg and Bang, 1975; Castelli et al., 1986; Stamler et al., 1986). Epidemiological studies have also found that in populations where CHD rates are high, such as North America, diets tend to be rich in total fat, saturated fat and cholesterol. Furthermore, numerous experimental studies have demonstrated that dietary saturated fatty acids (SFA) increase plasma cholesterol levels (Keys et al., 1957; Hegsted et al., 1965; Ginsberg et al., 1990; Barr et al., 1992) mainly by increasing plasma low

density lipoprotein cholesterol (LDL-C) which appears to be the most atherogenic lipoprotein (Grundy, 1987). On the other hand, unsaturated fatty acids (unSFA), both polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA), have been shown to decrease plasma cholesterol levels (Mattson and Grundy, 1985; McDonald et al., 1989; Ginsberg et al., 1990; Wardlaw and Snook, 1989; Mensink and Katan, 1989; Kestin et al., 1990; Chan et al., 1991; Barr et al., 1992). Recent studies (Mattson and Grundy, 1985; Ginsberg et al., 1990; Barr et al., 1992) reported that the mechanism by which PUFA and MUFA reduced plasma and LDL-C concentrations was merely by replacing SFA in the diet. Thus the quantity of SFA in the diet, rather than the quantity of total dietary fat, was found to be the key variable associated with high levels of plasma and lipoprotein (LP) cholesterol (Ginsberg et al., 1990; Barr et al., 1992).

The role of dietary fatty acids in thrombosis is less clear. Reports suggest that dietary SFA are thrombogenic compared to PUFA of the omega 6 (n-6) family, whereas PUFA of the n-3 family are antithrombogenic compared to the n-6 PUFA (Leaf and Weber, 1988; Hunter, 1991; Ulbricht and Southgate, 1991). Dietary n-6 PUFA, via their conversion to eicosanoids, are thought to influence the initiation and progression of atherosclerosis and thrombosis. Dietary n-3 PUFA appear to modulate eicosanoid synthesis and thus may have the potential for the amelioration of atherogenesis and thrombosis (Leaf and

Weber, 1988; Kinsella et al., 1990; Knapp, 1990). Linoleic acid (LA; 18:2n-6) and α-linolenic acid (LNA; 18:3n-3) are elongated and desaturated to arachidonic acid (AA; 20:4n-6) and eicosapentaenoic acid (EPA; 20:5n-3), respectively, by the same enzyme pathway (Brenner, 1989). Thus competition between dietary LA and LNA for elongation and desaturation processes may influence the fatty acid composition and prostanoid synthesis of various tissues and thus the risk of thrombus formation.

Previous work conducted in our laboratory (Chan, 1990) indicates that the absolute level of LNA in the diet influences the metabolism of n-6 fatty acids, whereas the relative amounts of LA and LNA, i.e. ratio of LA/LNA, influences n-3 metabolism in normolipidemic males. Budowski and Crawford (1985) also have suggested that the ratio of dietary LA/LNA may be more important than the absolute amounts of LNA in influencing LNA metabolism. Furthermore, many researchers are suggesting that the dietary LA/LNA ratio consumed by Western populations is too high and places n-3 PUFA metabolism at a disadvantage for mechanisms of desaturation and elongation (Budowski and Crawford, 1985; Nestel, 1987; Leaf and Weber, 1988; Nordoy and Goodnight, 1990; Kinsella, 1990). There is appreciable debate over the ability of dietary LNA to influence n-3 PUFA metabolism and research, to date, has not determined the absolute nor the relative amounts of LA and LNA required in the diet to reduce

the risk of developing CHD. Thus research which attempts to determine the relative amounts of LA and LNA needed in the diet may be very important to an understanding of CHD.

1.2 Pathology of Atherosclerosis

The pathogenesis of atherosclerosis has been described by several authors (Ross, 1986; Leaf and Weber, 1988; Zeman, 1983; Hornstra, 1989). A brief summary of the disease process is outlined in Figure 1. Atherosclerosis begins with damage to the endothelial lining of medium-sized arteries. The damage could be the result of a toxin, trauma or infection. Risk factors which often lead to lesion (atheroma) formation are smoking, obesity, hypertension, diabetes and high levels of LDL-C.

When damage occurs to the endothelium, endothelial cells are lost and monocytes and platelets adhere to the site of injury. Monocytes are attracted to the damaged endothelium and are activated to become scavenger cells called macrophages. Platelets exposed to the underlying connective tissue are also activated by the adhesion process and as a result, two other processses are started: the biosynthesis of platelet aggregating TXA_2 and the "release reaction" which leads to the release of several compounds by the thrombocyte (Hornstra, 1989). These compounds attract passing blood platelets which adhere to the already aggregated platelets. The newly aggregated platelets become activated and undergo

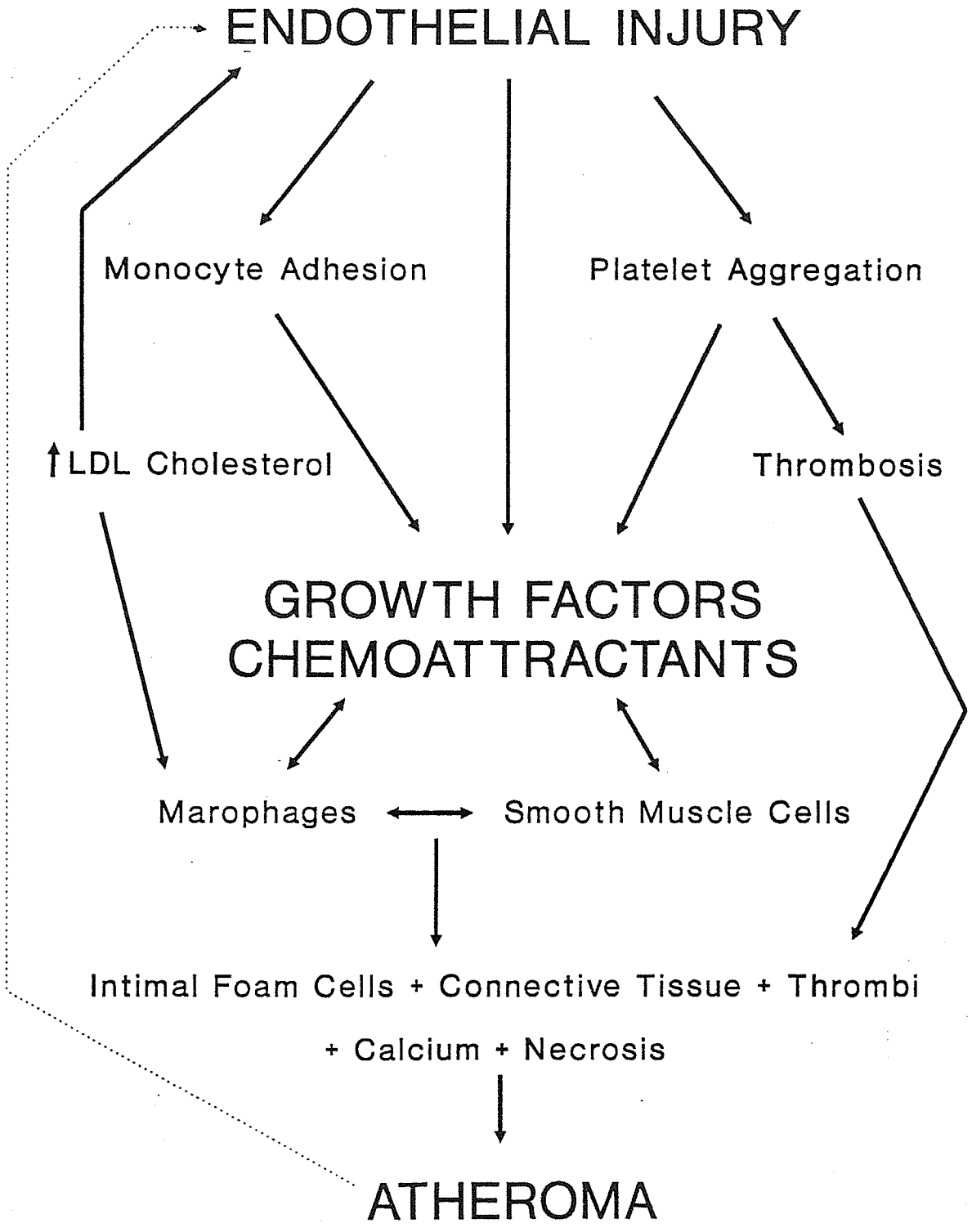


Figure 1. Pathogenesis of Atherosclerosis

the release reaction as well, thus forming a platelet thrombus at the site of injury. The platelet thrombus is stabilized by fibrin, formed as a result of the coagulation process, which is also initiated by vascular injury (Hornstra, 1989).

Activated platelets also release platelet-derived-growth-factor (PDGF) which binds to connective tissue sites of endothelial injury and attracts smooth muscle cells from the media of the vessel into the intima. Growth factors stimulate the proliferation of smooth muscle cells and macrophages around the injury site. Circulating LDL-C may be altered by phospholipase A_2 generated by endothelial cells, or it may be oxidized by macrophages in lesions through the non-scavenger LDL receptor pathway, forming a foam cell/fatty streak. The lipid accumulates extracellularly in the endothelium. The composition of the accumulated lipids has led researchers to believe it is derived from LDL (Zeman, 1983). If the oxidized LDL is toxic to the endothelium, the endothelial injury progresses from a fatty streak to a more advanced fibrous plaque and expands in size to narrow the lumen of the vessel. The overlying endothelium is further damaged setting up a viscious cycle. Generally, a thrombus finally occludes the vessel resulting in myocardial infarction (MI).

The development of atherosclerosis is a long-term or chronic process, while thrombus formation is a short-term or an acute incident (Hornstra, 1989; Kinsella, 1988; Truswell, 1985). The aggregation of blood platelets is involved in both

processes. Thus the tendency of blood platelets to aggregate is important in the development of atherosclerosis and thrombus formation. Since dietary fat is known to influence both processes, attention should be given to the role that dietary fatty acids may play in the prevention of CHD.

1.3 Dietary Fatty Acids and Atherosclerosis

1.3.1 The Occurrence of CHD: Epidemiological Studies and Dietary Intervention Studies

Epidemiological studies, carried out in many different parts of the world, have found correlations between the intake of SFA and dietary cholesterol and mortality from CHD. In the Seven Countries Study, the 15-year death rate indicated that mortality from CHD was negatively associated with the percentage of energy from dietary MUFA and was unrelated to the intake of PUFA. The dietary factor most closely correlated with high levels of plasma cholesterol and rates of CHD was the level of intake of dietary SFA (Keys, 1986). More recently, Dyerberg et al. (1986) reported another dimension in the relationship between dietary fats and CHD. They found that traditional Greenland Eskimos had a lower incidence of CHD than Eskimos living in Denmark despite the fact that the Eskimo diet was as high in fat and cholesterol as that of Danes or Americans. The Greenland Eskimo diet contained half the amount of SFA and LA, nearly five times as much n-3 PUFA, and nearly twice as much MUFA as the Danish diet.

Early dietary intervention studies, such as the Multiple Risk Factor Intervention Trial (MRFIT), revealed it was possible to reduce serum cholesterol concentrations by reducing the intake of SFA and increasing the intake of LA (Stamler et al., 1986). Men who later died from CHD and its complications had only small reductions in serum cholesterol, whereas those who survived had greater reductions in serum cholesterol. Hence their evidence suggested that reducing serum cholesterol concentration by dietary intervention could produce reductions in the CHD mortality rate.

In summary, epidemiological studies have demonstreated a positive correlation between a high intake of SFA and CHD and a negative correlation with a high intake of n-3 fatty acids from fish. In addition, dietary intervention studies indicated that it is possible to reduce plasma cholesterol levels by replacing dietary SFA with LA and that the reduction in plasma cholesterol levels is conducive to a decrease in CHD.

1.3.2 The Effects of SFA, MUFA, and PUFA on Plasma Lipids and Lipoproteins

1.3.2.1 SFA

There is a wealth of evidence indicating that a high dietary intake of SFA increases total cholesterol (TC) levels (Keys et al., 1957; Hegsted et al., 1965), mainly by increasing LDL-C levels (Ginsberg et al., 1990; Barr et al.,

1992). Keys et al. (1957) derived a multiple regression equation to explain the observed effects of different types and amounts of fats on changes in serum cholesterol levels in men. They concluded that SFA (gram-for-gram) were about twice as effective in raising serum cholesterol as PUFA were in reducing serum cholesterol and that MUFA had little, if any effect. Later, Keys modified this formula to include lauric, myristic and palmitic acids (12:0, 14:0, 16:0, respec.) as the only hypercholesterolemic SFA (Grande et al., 1963). Hegsted et al. (1965) subsequently reported that approximately 67% of the total variance in the level of serum cholesterol was explained by myristic acid and that palmitic acid (PMA) had a significant but lesser effect on serum cholesterol.

The mechanisms by which SFA are believed to increase plasma cholesterol levels have been under much debate. One mechanism which has received considerable support on the basis of animal and human studies is that dietary SFA acts similarly to dietary cholesterol by increasing the hepatic content of cholesterol, which in turn suppresses the activity of LDL receptors, and thus produces an increase in plasma cholesterol levels (Spady and Dietschy, 1988; Grundy, 1985; Grundy, 1986; Grundy, 1987). However, the intracellular mechanisms responsible for this action have not been identified and studies have not provided conclusive evidence to indicate whether SFA act on the metabolism of cholesterol or on the function of the LDL receptors on the surface of the cells

(Grundy, 1987).

Recent investigations confirm the results of earlier studies with the addition of some new findings. For instance, myristic acid is believed to have approximately four times the atherogenic potential of PMA. By contrast, stearic acid (STEA; 18:0) has shown little, if any, atherogenic effects although it may promote thrombogenesis (Ulbricht and Southgate, 1991). In the light of these findings, Ulbricht and Southgate (1991) proposed that the ratio of PUFA to SFA (P/S ratio), often used to measure the atherogenicity of a diet, should be replaced by indices of atherogenicity and thrombogenicity since SFA differ in their effects on hypercholesterolemia and thrombogenicity. Other researchers agree that the P/S ratio of the diet is obviously inappropriate in determining the atherogenicity of a diet (Leaf and Weber, 1988; Kinsella, 1988).

1.3.2.2 MUFA

At one time MUFA were considered neutral, i.e. they neither raised nor lowered plasma lipids (Keys et al., 1957). However, the Seven Countries Study also found that the incidence of CHD was low in the Mediterranean countries even though the fat intake of the population was high (Keys et al., 1986; Grundy, 1987). The main dietary fat was olive oil which is rich in oleic acid (OA; 18:1n-9). This observation led to an interest in studying the effects of OA on plasma

cholesterol levels.

Many studies have compared the effects of OA to LA as a replacement for SFA. Evidence indicates that diets high in OA were as effective as diets high in LA in reducing TC and LDL-C, but in contrast to the effects of n-6 PUFA, OA did not lower high density lipoprotein cholesterol (HDL-C) (Mattson and Grundy, 1985; Mensink and Katan, 1989; Chan et al., 1991; McDonald et al., 1989). The LDL-C/HDL-C ratio, an important risk factor for atherosclerosis (Nordoy and Goodnight, 1990), was significantly lower following the OA-containing diet than the ratio produced by control diets rich in LA or by a low-fat (20% of energy) diet (Grundy, 1987; Grundy, 1986). In fact, the diet rich in OA was superior to the low-fat high carbohydrate diet in lowering the LDL-C/HDL-C ratio. The high carbohydrate diet raised triglyceride (TG) levels and decreased HDL-C, whereas the OA diet had no effect on either of these blood constituents (Grundy, 1986). Furthermore, Ginsberg et al. (1990) demonstrated that the addition of OA to an American Heart Association (AHA) Step 1 diet did not alter the benefits of the Step 1 diet, namely a reduction in plasma TC and LDL-C. Neither the high OA or Step 1 diet had any effect on HDL-C or plasma TG in these studies. Thus the literature suggests that diets rich in OA are as effective as diets rich in LA in reducing TC and LDL-C.

The mechanism responsible for the hypocholesterolemic response of dietary OA is not clear. Mattson and Grundy

(1985) reported that the composition of LDL did not change when OA was substituted for SFA in the diet. Therefore, the fall in plasma LDL levels were not due to the depletion of LDL of cholesterol. On the other hand, it is possible that MUFA may enhance the activity of LDL receptors. However, it is also possible that the rise in LDL receptor activity following an OA diet may simply be due to the removal of the suppressive action of dietary SFA.

1.3.2.3 n-6 PUFA

LA, derived from vegetable seed oils, is a major constituent of corn, soybean, cottonseed and sunflower oils and is the parent member of the n-6 family of fatty acids. Although the Seven Countries Study failed to demonstrate a correlation between the intake of dietary PUFA and a reduced mortality rate from CHD (Keys et al., 1986), the hypocholesterolemic effect of LA is very well established in human studies. The main effect of LA-containing oils is to lower LDL-C concentrations (Chan et al., 1991; McDonald et al., 1989; Mattson and Grundy, 1985; Grundy, 1987; Goodnight et al., 1982). Thus dietary recommendations have focused mainly on replacing SFA with LA (Grundy, 1987; Ulbricht and Southgate, 1991; Nordoy and Goodnight, 1990). However, there is evidence that LA may also lower HDL-C levels, especially when consumed in large amounts (Mattson and Grundy, 1985; Mensick and Katan, 1989; Goodnight et al., 1982). In some

studies where HDL-C was reduced, similar or even larger decreases in LDL-C concentration occurred which led to a constant LDL-C/HDL-C ratio (Goodnight et al., 1982). Reductions in HDL-C levels have been of concern since HDL-C levels have been inversely related to CHD (Castelli et al., 1986; Grundy, 1987). HDL is believed to be involved in the removal of cholesterol from extrahepatic tissues and its return to the liver for recycling or disposal. For these reasons, dietary recommendations now suggest that PUFA intake should not exceed 15% of total energy (Beynen and Katan, 1989) and that the P/S ratio of the diet should not be > 1.0 (McNamara, 1991).

However, there is no evidence to suggest that reductions in HDL-C concentrations accompanied by a proportional decrease in the LDL-C concentration is harmful; populations with low CHD rates frequently have lower HDL-C levels than those in Western countries with high rates of CHD (Nestel, 1987). Recent controlled metabolic studies indicate that significant reductions in plasma HDL-C levels did not occur with modestly high LA intakes (McDonald et al., 1989; Chan et al., 1991; Wardlaw and Snook, 1989; Goodnight et al., 1982). Furthermore, Nestel (1987) reported that low-fat diets (20% of energy or less) lower both LDL-C and HDL-C levels, whereas a modest restriction of fat intake (30% of energy) with a P/S ratio of 1 generally lowers LDL-C without affecting HDL-C levels.

There has been considerable debate over the mechanism by which LA reduces plasma and LP cholesterol levels. Results from a number of studies have been variable and inconsistent in their findings. It was once thought that LA had a unique cholesterol-lowering effect that extended beyond simply its replacement for dietary SFA (Keys et al., 1957; Hegsted et al., 1965). However, it is unlikely that the composition and structure of LDL are altered by LA rich diets, although this may be dependent on the degree of LA enrichment (Nestel, 1987; Vega et al., 1982). Recent results from carefully controlled studies indicate that LDL-C levels are reduced only when unSFA are substituted for SFA in the diet (Grundy, 1987; Ginsberg et al., 1990; Barr et al., 1992).

There has been some concern over the idea of high intakes of LA. This is supported by the fact that no major population group has consumed large quantities of PUFA with proven safety (Nestel, 1987; Grundy, 1987). In addition, there have been reports that LA can promote the development of tumors in laboratory animals treated with carcinogens and that LA may suppress the immune system (Nestel, 1987). For these reasons, investigators are interested in studying the effects of other fatty acids such as OA and n-3 PUFA in the events predisposing to CHD.

1.3.2.4 n-3 PUFA

LNA, the parent member of the n-3 family of PUFA, occurs primarily in the chloroplast membrane of plants rather than in the seed oils (Nestel, 1987). Vegetable oils such as canola and soybean oil contain approximately 10% and 8% of total fatty acids as LNA, respectively, whereas linseed (flax) oil contains approximately 55% LNA (Goodnight et al., 1982). Currently it is estimated that the Canadian intake of LNA is about 2 g/day, mainly in the form of canola oil (Hunter, 1990). Long chain (LC) n-3 PUFA, EPA and DHA, can be obtained directly from the diet but the only concentrated sources are from fish and the oils derived from them.

In early studies, Grande et al. (1963) reported that fish oils did not appear to be more hypocholesterolemic than oils containing predominantly LA. However, Dyerberg and Bang (1986) later reported that Greenland Eskimos, eating a traditional marine diet, had significantly lower TC, LDL-C, very low density lipoprotein cholesterol (VLDL-C) levels and higher HDL-C levels than Eskimos living in Denmark and Danes of all ages and both sexes. Numerous experimental studies have since attempted to determine the effects of fish and fish oils on the plasma lipids and LP in humans. It is currently believed that n-3 PUFA are not as equally effective in reducing TC levels as n-6 PUFA. The reduction in plasma cholesterol following n-3 PUFA supplementation is largely due to a reduction in VLDL-C levels accompanied by a more

pronounced reduction in TG levels than with n-6 PUFA (Beynen and Katan, 1989; Nestel, 1987). Harris et al. (1983) found that a safflower-corn oil diet and a salmon oil diet were equally effective in reducing plasma cholesterol but the vegetable oil was not as effective as the salmon oil diet in reducing plasma TG. By contrast, several studies report that fish oils increased LDL-C levels while confirming that fish oils were much more effective than LA-containing oils in reducing plasma VLDL-C and TG (Kestin et al., 1990; Harris, 1989). Harris (1989) suggested that these discrepancies may be because in the majority of studies reporting decreases in LDL-C, SFA intake was reduced on the experimental diets (i.e., fish oil diet) and thus the reduction in SFA may be responsible for decreased LDL-C levels. However, recent reviews (Harris, 1989; Kinsella, 1981) conclude that trials using smaller, more practical doses of fish oils rather than pharmacological doses, find fish oils to exert their most dramatic effects by reducing VLDL-C and plasma TG levels while slightly increasing HDL-C levels (5-10%) in normal and hypertriglyceridemic subjects.

On the other hand, dietary LNA has not been studied as extensively as fish oils and adequate comparisons have not been made between the cholesterol reducing potential of LNA and fish oils. Some reports have suggested that dietary LNA is not as effective in reducing plasma cholesterol levels as preformed EPA and DHA (Nettleton, 1991; Sanders and Roshanai,

1983). For example, Sanders and Roshanai (1983) compared the effects of a linseed oil supplement to a MaxEPA (fish oil concentrate) supplement on the plasma lipids and LP in five healthy subjects. They found that a 20 mL/day linseed oil supplement did not significantly alter plasma cholesterol levels, but a 10 g MaxEPA supplement decreased plasma TG and a 20 g MaxEPA supplement decreased plasma TG, TC and HDL-C levels. However, their findings are compromised by the failure to control the dietary fat intake of the subjects, thus making it difficult to extrapolate their results. In a carefully controlled study, Kestin et al. (1990) compared the lipid lowering effects of linseed oil (9.2 g/day) and MaxEPA (3.4 g/day) to safflower oil (14.3 g/day) in 33 mildly hypercholesterolemic men. Small reductions in TC and LDL-C levels occurred in response to the safflower oil and linseed oil diets but not in response to the MaxEPA diet. MaxEPA lowered VLDL-C, increased LDL-C and had little effect on HDL-C levels. Hence, LNA appeared to be as effective as LA in reducing LDL-C levels but the fish oils were more effective in reducing VLDL-C and TG levels. Valsta et al. (1992) compared the effects of low erucic rapeseed oil (canola oil) to the effects of sunflower oil on the plasma lipoprotein levels of 59 subjects. The results indicated that the rapeseed oil diet reduced LDL-C levels more than the sunflower oil diet, whereas the sunflower oil diet was more effective at reducing VLDL-C and TG levels than the rapeseed oil diet. Furthermore,

Lasserre et al. (1985) found that long-term consumption (5 months) of a rapeseed oil diet led to decreases in TC levels but did not affect TG levels. Thus, the evidence suggests that LNA-containing oils, in contrast to fish oils, do not necessarily exert their lipid-lowering effects through a reduction in VLDL-C and TG levels but may be more effective in reducing LDL-C levels than fish oils.

Previous studies in our laboratory (Chan et al., 1991; Chard, 1991) indicate that LNA is equally hypocholesterolemic to dietary LA and OA without affecting HDL-C concentrations in both normal and hyperlipidemic subjects, respectively. Similarly, McDonald et al. (1989) found that canola oil was as effective as sunflower oil in reducing TC and LDL-C levels when fed to eight normolipidemic men for 42 days. The cholesterol-lowering effects of dietary LNA from oils such as canola oil and linseed oil have not been demonstrated as frequently as those of dietary LA and OA. However, evidence from carefully controlled studies conducted in our laboratory, and others, support the hypocholesterolemic effects of LNA. The mechanism by which dietary LNA reduces plasma and LP cholesterol levels is assumed to be similar to the that of LA and OA, which is by merely replacing dietary SFA.

LNA-containing oils may be more susceptible to oxidative change than LA and OA-containing oils due to their high degree of unsaturation. Thus the introduction of low linolenic (low-LNA) canola oil has created interest for use by the food

service and food manufacturing industries. However, there is no data which documents the nutritional properties of low LNA canola oil. Low-LNA canola oil, which contains more OA and LA than traditional canola oil, would be expected to affect plasma cholesterol levels similarly to traditional canola oil. However, previous work from our labratory indicates that low-LNA canola oil may differ from traditional canola oil with respect to its effects on thrombogenesis. Hence there is a need to compare both the atherogenic and thrombogenic effects of low-LNA canola oil to those of regular canola oil.

1.4 Dietary Fatty Acids and Thrombosis

1.4.1 Pathways of Fatty Acid Metabolism and Prostanoid Synthesis

LNA metabolism follows several pathways: oxidation, elongation and desaturation, incorporation into PL or cholesterol esters, and prostanoid synthesis (Zollner, 1986). The conversion of LNA to its LC derivatives is produced by alternating sequences of desaturation and elongation (Figure 2). The same enzymes, located in the endoplasmic reticulum membrane, produce the desaturation and elongation of LA and LNA (Brenner, 1989). The rate-limiting step in these reactions, located at the beginning of the biosynthetic scheme, is the Δ6-desaturase reaction. This enzyme is sensitive to product feedback inhibition by LC fatty acids; to competitive inhibition by other PUFA; and has a greater

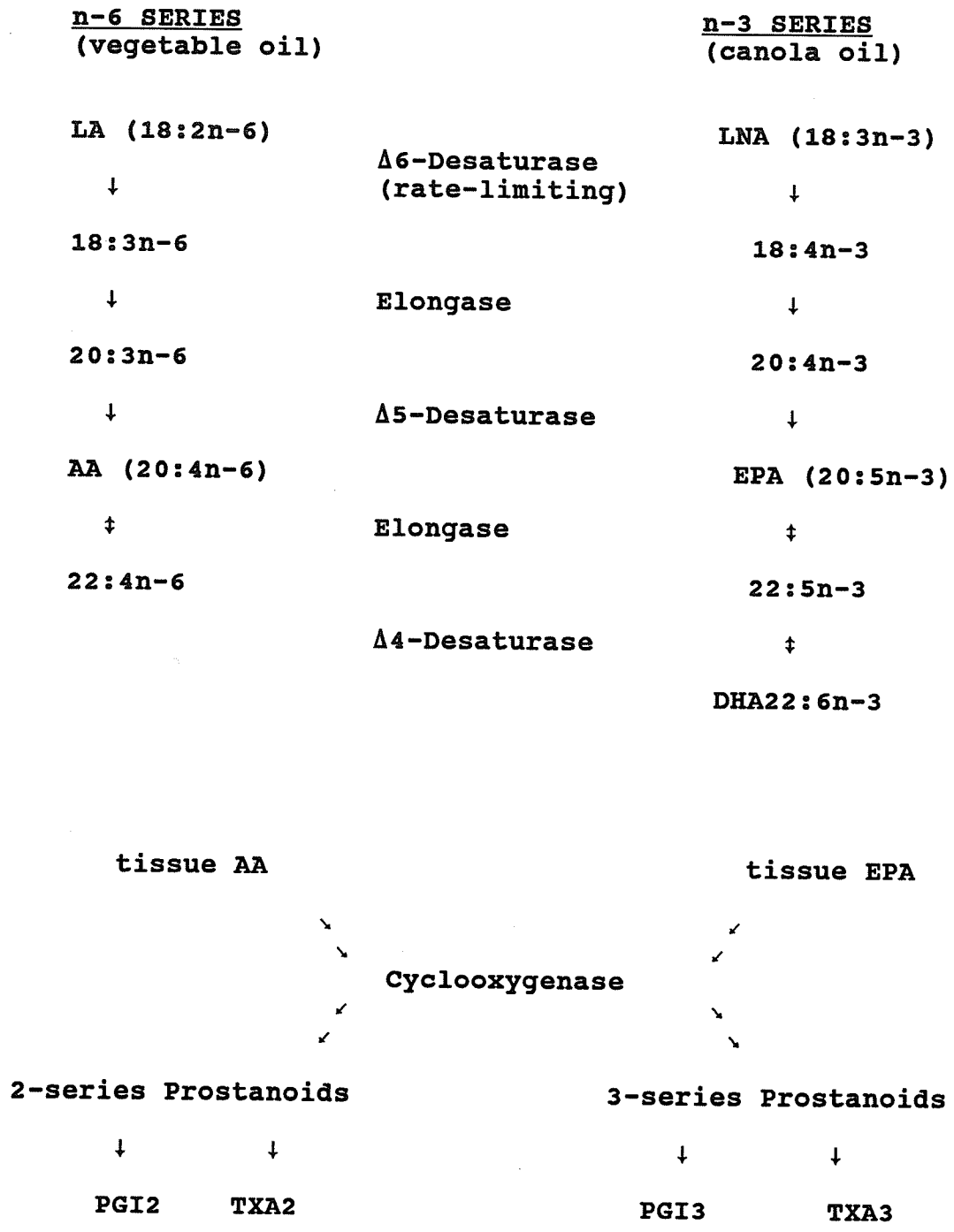


Figure 2. Enzyme Pathways of Fatty Acid Desaturation and Elongation and Prostanoid Synthesis

affinity for LNA than LA (Brenner, 1989). Thus one would expect dietary LNA, in the presence of LA, to be converted to EPA and DHA while inhibiting the metabolism of LA to AA.

Dietary and endogenously produced AA and EPA are esterified and incorporated into the ß-position of PL in biomembranes (Vergroesen, 1989). The synthesis of prostanoids, a group of eicosanoids which includes the prostaglandins and thromboxanes, is dependent on the availability of EPA and AA stored mainly in membrane PL. Prostanoids are formed using free fatty acids (FFA). Thus EPA and AA must be mobilized by phospholipase A_2, stimulated in response to injury and inflammation, before they can be converted into prostanoids (Crawford, 1989). Under normal conditions, neutral lipids, derived directly from the diet and present in circulating pools, may also provide small amounts of FFA for eicosanoid synthesis (Crawford, 1989). AA and EPA also compete for the key enzyme, cyclooxygenase, involved in prostanoid synthesis (Hornstra, 1989). Thus the amounts of these fatty acids available for prostanoid formation are dependent upon the amounts of EPA and AA in membrane lipids and in turn, the amount of LA and LNA provided by the diet for the competition of Δ6-desaturase.

To briefly summarize prostanoid formation (Figure 2), LA is converted to AA which is the precursor of thromboxane A_2 (TXA_2) and prostacyclin (PGI_2), whereas LNA is converted to EPA which is the precursor of TXA_3 and PGI_3. PGI_2 and PGI_3,

produced by the endothelium of blood vessels, are vasodilatory and platelet antiaggregatory. TXA_2, produced by blood platelets, is a strong vasoconstricting and platelet aggregating agent whereas TXA_3 is a weak platelet aggregator (Kinsella, 1988; Leaf and Weber, 1988). The production of TXA_2 must be balanced by the production of PGI_2 to maintain vasal tone and blood circulation. An excess of TXA_2 is conducive to platelet aggregation and possibly thrombus formation. The reduction of AA or its substitution with EPA is an approach for ameliorating this situation (Kinsella, 1988). Thus if dietary LNA competes favorably for Δ6-desaturase and increases the EPA content in PL, then prostanoid homeostasis should shift towards a less thrombogenic state.

1.4.2 Epidemiological Studies and Dietary Intervention Studies in Thrombosis

In general, results from epidemiological studies indicate that the ingestion of LNA or EPA results in higher concentrations of LC n-3 fatty acids in plasma and platelet PL and a reduction in the thrombogenic tendency of blood platelets, possibly through altered eicosanoid synthesis (Dyerberg and Bang, 1979; Knapp, 1988). These observations prompted a great deal of interest in the role of n-3 PUFA in relation to thrombosis.

Dyerberg and Bang (1979) found that traditional Greenland

Eskimos, who consumed high amounts of dietary EPA and DHA from fish, had plasma and platelet lipids enriched with EPA and DHA. The Eskimos also had significantly longer bleeding times and platelet aggregation was inhibited compared to Danes and Eskimos living in Denmark. Further analyses indicated that the Greenland Eskimos had altered eicosanoid profiles; that is, they excreted higher quantities of PGI_3 and PGI_2 metabolites and lower quantities of TXA_2 and TXA_3 metabolites in their urine than a similar group of Danes. In a dietary intervention study, Renaud et al. (1986) found that long-term low intakes (1% of calories) of LNA by French farmers were effective in altering n-6 and n-3 LC PUFA incorporation into plasma and platelet PL when LA intake was restricted to 5% of calories. Levels of EPA increased and levels of AA decreased in plasma and platelet PL. In addition, in vitro clotting activity and platelet aggregation to thrombin were inversely related to LNA intake. These results suggested that small changes in the levels of EPA and AA in PL, due to slight dietary modifications of LNA and LA, altered platelet clotting activity and thus had a beneficial role in thrombogenesis.

1.4.3 LNA Metabolism and Thrombosis

There is evidence that dietary LNA, in contrast to LA, is not as efficiently incorporated into plasma and membrane lipids as its long chain homologs, EPA and DHA (Budowski and Crawford, 1985; Nettleton, 1991; Adam et al., 1986; Zollner,

1986; Dyerberg, 1986; Sanders and Younger, 1981; Sanders and Roshanai, 1983). However, LNA-containing diets have been reported to increase the LNA and EPA content of plasma and platelet PL (Chan, 1990; Corner et al., 1990; Chard, 1991; Weaver et al., 1990; Mest et al., 1983; Adam et al., 1986; Sanders and Roshanai, 1983; Sanders and Younger, 1981; Renaud et al., 1986; Lasserre et al., 1985; Budowski et al., 1984). Increases in the content of EPA of PL suggests that dietary LNA can be elongated and desaturated to EPA in the human body since EPA was not present in the diets. Although, the conversion of LNA to EPA is thought to be slow in humans (Leaf and Weber, 1988; Nettleton, 1991; Zollner, 1986).

Adam et al. (1986) studied the effect of dietary LNA on the composition of plasma and platelet PL. Formula diets containing four different levels of LNA (0-16% of total calories) were fed to six healthy women. The results demonstrated that LNA was incorporated into the PL of human plasma and platelets in a dose-dependent manner. The incorporation of LNA occurred at the expense of OA, not at the expense of LA. However, the increase in plasma and platelet LNA was much smaller than analogous experiments with LA (Zollner, 1986), suggesting that LNA may not be incorporated into PL as efficiently as LA. Furthermore, the amount of EPA incorporated into plasma and platelet PL was low, suggesting that the conversion of LNA to EPA was slow since dietary EPA itself is readily incorporated into tissue PL (Zollner, 1986).

Sanders and Roshanai (1983) investigated the effect of dietary LNA on LA metabolism in five subjects receiving either a 20 mL linseed oil or MaxEPA supplement daily for two weeks. The EPA content of platelet PL was increased by the linseed oil supplement, but to a much lesser extent than by the fish oil supplement. Platelet AA level decreased on the MaxEPA supplement but not on the linseed oil supplement suggesting that the linseed oil diet did not increase platelet EPA levels enough to displace platelet AA levels. However, the significance of these results is difficult to determine because the intake of dietary fat was not controlled during the experimental periods.

By contrast, Budowski et al. (1984) found that plasma LNA and EPA increased slightly (>2%), whereas LA and AA decreased when healthy subjects were given a 60 mL/day linseed oil supplement for six weeks. In addition, platelet aggregation to collagen decreased after linseed oil supplementation. Similarly, Mest et al. (1983) found that administering a 30 mL/day linseed oil supplement to ten volunteers for four weeks resulted in higher levels of LNA, EPA and DHA in plasma PL. These changes occurred at the expense of LA and AA, which decreased during the linseed supplementation.

Studies using preformed EPA and DHA indicate that EPA and DHA may be more effective sources of LC n-3 PUFA than LNA for replacing LC n-6 PUFA in PL pools (Leaf and Weber, 1988; Sanders and Younger, 1981; Sanders and Roshanai, 1983;

Kinsella, 1988; Nettleton, 1990). Consequently, there is some debate as to the usefulness of LNA as a source of LC n-3 PUFA and more research is required to determine the effectiveness of dietary LNA, particularly when intakes of LA are high. Nevertheless, dietary LNA is conceivably a more important source of LC n-3 PUFA for populations not accustomed to diets containing fish or fish oils. Furthermore, most studies examining the effects of fish oils on PL composition have administered pharmacological doses rather than practical doses. Thus few studies have demonstrated the effectiveness of dietary EPA supplied at low doses and few studies have adequately compared the effects of dietary LNA to small doses of EPA and DHA. In addition, there are some reported adverse effects associated with a high consumption of fish oils which need to be considered, such as: prolonged bleeding, reduced immune response, and vitamin A and D toxicity (Leaf and Weber, 1988).

Furthermore, the equivocal results in the literature may indicate the need for higher dietary levels of LNA or for long-term intakes of LNA, especially for subjects consuming high amounts of LA (Kinsella, 1988). Lasserre et al. (1985) found that long-term consumption of a diet providing 4.5% of total energy as LA and 1.5% as LNA increased the LNA and EPA content of serum PL and CE but was not effective in reducing LA and AA levels. In fact, a diet containing 6.5% of total energy as LA and negligible amounts of LNA had the same effect

on LC n-6 PUFA levels as the diet containing 1.5% LNA. Therefore, a long-term low intake of dietary LNA (1.5% of total energy) had no suppressive effect on LC n-6 PUFA levels when the LA supplied 4.5% of total energy. Thus, the beneficial effects of LNA intake may be negated by the amount of LA in the diet.

1.4.4 Importance of the Dietary LA/LNA Ratio

Currently there is debate over the importance of the dietary LA/LNA ratio in influencing the amount of LC n-3 PUFA incorporated into plasma and tissue PL. In rat trials, linseed oil diets provided a reduction in the proportion of LA to LNA in the diet and improved the efficacy of LNA to synthesize EPA and DHA while decreasing AA in plasma and tissue PL, plasma CE and TG (Christiansen et al., 1990; Engler et al., 1991; Marshall and Johnston, 1982; Huang et al., 1986; Lee et al., 1989; Ishinaga et al., 1989). A reduction in collagen-induced platelet aggregation and eicosanoids of the two-series accompanied the fatty acid compositional changes (Ishinaga et al., 1989; Marshall and Johnston, 1982). However, due to species differences, animal models do not provide adequate information regarding the desirable levels of dietary LNA and LA required for optimal membrane conditions in humans (Budowski and Crawford, 1985).

In several human studies, increasing the LNA content of the diet increased the EPA content of PL minimally and did not

affect the LA or AA content of plasma and platelet PL (Adam et al., 1986; Sanders and Roshanai, 1983; Lasserre et al., 1985; Chan, 1990; Corner et al., 1990; Weaver et al., 1990). Adam et al. (1986) found that only small amounts of LNA and EPA were incorporated into platelet PL and neither plasma nor platelet AA levels were altered with diets containing a constant amount of LA (4% of total calories) but varying amounts of LNA (0-16% of total calories). By contrast, Sanders and Younger (1981) found that the LA/LNA ratio of the experimental diets did affect LA and LNA metabolism. They administered a linseed oil supplement (20 mL/day) to vegan and omnivore subjects for two weeks. The supplements together with the subjects' habitual diets provided dietary LA/LNA ratios of 1/1 and 3/1, respectively. Mean EPA level increased two-fold (from 1.3% to 2.7%) in plasma phosphatidylcholine (PC) of the omnivores and three-fold (from 0.3% to 1.0%) in the plasma PC of the vegans. On the other hand, although the level of EPA in total platelet PL also increased two-fold (0.6% to 1.2%) during linseed oil supplementation in the omnivores, no change occurred in the vegans (0.3%). Linseed oil supplementation had no effect on plasma and platelet DHA levels in either group. In addition, the levels of plasma and platelet EPA and DHA were appreciably lower and the levels of LA and AA higher for the vegans than the omnivores both before and after LNA supplementation. The authors suggested that a longer period of supplementation may have produced a greater

increase in platelet and plasma EPA because the high tissue levels of LA in the vegans may have affected LNA metabolism.

Corner et al. (1990) and Weaver et al. (1990) found significantly higher LNA and EPA levels in plasma and platelet PC and alkenylacyl ethanolamine phosphoglyceride (PPE) fractions following a canola oil diet compared to a sunflower oil diet (LA/LNA ratios: 2.6/1 and 73.9/1, respec.) in normolipidemic males. AA levels were lower in the plasma phosphatidylethanolamine (PE) and plasma PPE (Corner et al., 1990) and platelet PC fractions (Weaver et al., 1990) following the canola diet. The results suggested that EPA selectively replaced some of the AA in plasma and platelet PL. In other words, the fatty acid composition of the PL fractions responded differently to LNA dietary treatment and there were differences in the fatty acid incorporation between fractions in the plasma and platelet PL. Furthermore, McDonald et al. (1989) found that the canola oil diet significantly increased the bleeding time and in vivo 6-keto-$PGF_{1\alpha}$ production, the stable inactive metabolite of PGI_2, whereas the sunflower oil diet did not. Thus, the results indicated that higher EPA levels in plasma PL in response to a canola diet may result in longer bleeding times and altered eicosanoid metabolism in heathly subjects. Chan et al. (1993) investigated the effects of diets containing LA/LNA ratios of 2.7/1, 3/1, 6.5/1 and 27.4/1 on the composition of plasma and platelet PL in healthy males. Significantly higher levels of LNA, EPA and n-3 PUFA

were incorporated into plasma PL following the diets containing low LA/LNA ratios (i.e., 2.7/1 and 3/1). However, dietary fatty acid composition had little effect on the AA content of PL, except for slightly lower levels in plasma PC. No differences in bleeding time and the production of TXB_2 were found among the diets although the production of 6-keto-$PGF_{1\alpha}$ and the 6-keto-$PGF_{1\alpha}/TXB_2$ ratio were significantly higher following the diet with the lowest LA/LNA ratio (2.7). Chan et al. (1993) also found that LC n-3 fatty acids were selectively incorporated into the PL fractions. Both Chan et al. (1993) and Weaver et al. (1990) reported a large increase in the amount of EPA incorporated in the PPE fraction of plasma and platelet PL. Holub et al. (1988) also reported that the major reservoir of EPA was in the PPE fraction of PL, whereas DHA was equally distributed in the PE and PPE fractions. Mori et al. (1987) reported that levels of EPA and DHA increased in platelet diacyl PC and PE fractions following MaxEPA supplementation. However, these researchers did not study the levels of LC n-3 PUFA in the PE and PC sub-classes.

Kinsella (1988) suggested that PUFA should provide <8% of total energy in the diet at a n-6/n-3 ratio of approximately 3/1. By contrast, Budowski and Crawford (1985) suggested that a LA/LNA ratio of approximately 5/1 is "natural" for humans since evolving man consumed a diet of these proportions. These ratios are far below the ratios of Western diets which are approximately 10/1-14/1 (Kinsella, 1988; Budowski and

Crawford, 1985; Nestel, 1987). It is possible that the quantity of LNA needed for optimum n-3 metabolism might be quite low at low intakes of LA. Thus at reduced intakes of n-6 PUFA, LNA provided by such oils as canola might be effective sources of n-3 PUFA. The work of Renaud et al. (1986), supports the idea that long-term low intakes of LNA, at relatively low intakes of LA, may be beneficial in platelet function. Because LA and LNA compete for the same desaturation and elongation system and for incorporation into tissue PL, the relative amounts of LA and LNA rather than the absolute amount of LNA in the diet may be more important for n-3 PUFA metabolism (Chan, 1990; Budowski and Crawford, 1985), and may also be important for n-6 PUFA metabolism.

1.5 Rationale for the Present Study

Dietary fatty acids influence the risk of developing CHD through their effects on blood cholesterol and by their ability to influence the thrombotic tendency of blood platelets (Ulbricht and Southgate, 1991). The hypercholesterolemic nature of dietary SFA and the hypocholesterolemic nature of dietary OA and LA are well known. The hypocholesterolemic effect of dietary LNA has also been shown in studies using linseed oil (McDonald et al., 1990; Chan et al., 1991; Kestin et al., 1990) and canola oil (McDonald et al., 1989; Chan et al., 1991; Wardlaw et al., 1991; Lassere et al., 1986; Renaud et al., 1986; Valsta et

al., 1992), which is characterized by relatively high levels of OA and LNA. The cholesterolemic effect of low-LNA canola oil, however, has not been documented, and thus should be confirmed.

Less is known about the thrombogenic effects of dietary fatty acids. Dietary SFA have been shown to be thrombogenic while PUFA and MUFA are said to be antithrombogenic. However, it is the n-3 PUFA family of fatty acids that are considered to be the most antithrombogenic unSFA. The antithrombogenic effects of fish oils have received considerable attention, while less information is available concerning the antithrombogenic effects of dietary LNA. The mechanism by which LNA and LC n-3 PUFA are thought to be involved in thrombosis is through the replacement of AA by EPA in PL and by the inhibition of AA-derived eicosanoid sythesis.

Dietary LA and LNA share the same enzymatic pathway of fatty acid desaturation and elongation. Therefore, the absolute and relative amounts of LA and LNA may influence the amounts of AA and EPA available for incorporation into the 2-acyl position of PL and for prostanoid synthesis. In situations where dietary LNA competes favourably with dietary LA for the Δ6-desaturase enzyme, EPA may be synthesized and incorporated into PL at the expense of AA, and thereby possibly reduce the synthesis of prostanoids from AA. When EPA replaces AA in platelet membranes, the amount of TXA_2 produced by blood platelets is suppressed while the amount of

PGI_2 synthesized by blood vessels is moderately suppressed (Dyerberg and Bang, 1986; Knapp, 1990). Instead, PGI_3 and TXA_3 are produced from their precursor, EPA, which results in a reduced thrombotic tendency of blood platelets (Hornstra, 1989).

Chan (1990) suggested that the absolute amount of dietary LNA may be more important in influencing n-6 PUFA metabolism while the LA/LNA ratio may be more important in influencing n-3 PUFA metabolism. In her studies, however, both the level of LNA and LA were varied in order to achieve different dietary LA/LNA ratios. Nevertheless, her findings emphasized the possible importance of the LA/LNA ratio of dietary fat and has created the need to further examine the significance of the dietary LA/LNA ratio in thrombosis. Both Corner et al. (1990) and Chan et al. (1993) found that dietary LNA increased the incorporation of LNA and EPA into plasma PL fractions when the dietary LA/LNA ratio was low (< 3.0). Meanwhile, Holub et al. (1988) reported that the major PL reservoirs of EPA, AA, docosapentaenoic acid (DPA; 22:5n-3), and DHA are in the PC, PE, and PPE fractions of platelets. Hence, studies which examine the effect of dietary LNA on tissue n-3 fatty acid composition should assess the fatty acid composition of these particular PL fractions.

The current study was undertaken to assess the effects of a low-LNA canola oil on plasma lipids (viz., cholesterol and triacylglycerol levels) and LP (LDL, HDL and VLDL levels).

In addition, the study was designed to determine the importance of the level of dietary LNA on the incorporation of LC PUFA into platelet PC, PE and PPE fractions when the level LA in the diet was held constant (i.e., when LNA levels and LA/LNA ratios were the primary variables).

2. OBJECTIVES

Low-LNA canola oil was developed by the plant breeder to improve the storage qualities of regular canola oil. However, it is not known if low-LNA canola oil shares the beneficial nutritional properties, with respect to fatty acid metabolism, of regular canola oil. Thus the primary objective of the present study was to compare the effect of low-LNA canola oil on plasma lipids, LP and the fatty acid composition of platelet PL with the effect of regular canola oil on these parameters in normolipidemic subjects. The secondary objective was to assess the importance of the dietary LA/LNA ratio on the plasma lipids, LP and fatty acid composition of platelet PL where the LA/LNA ratios of the diets were manipulated. The rationale behind the second objective stems from previous work in our labratory which indicated that the dietary LA/LNA ratio influenced the fatty acid composition of plasma PL and eicosanoid metabolism. In the earlier studies, the levels of both dietary LA and LNA were varied. In the present study, the level of LA in the diets remained constant while the level of LNA was varied in order to determine the relative importance of the LA/LNA ratio and of the amount of dietary LNA independent of differences in the level of dietary LA. Thus, the objectives of the present study were to determine:

OBJECTIVES (cont'd).

(i) the effect of low-LNA canola oil on the metabolism of n-3 amd n-6 fatty acids in a group of normolipidemic subjects; and

(ii) the effect of the dietary LA/LNA ratio and the level of dietary LNA on the fatty acid composition of platelet PL (viz., PC, PE and PPE) in a group of healthy male subjects.

3. MATERIALS AND METHODS

3.1 Experimental Design

The experimental model used in the 61-day study was a balanced incomplete randomized block design (BIBD). The first 7 days of the study served as a stabilization or pre-experimental period where all subjects received a mixed fat or control diet (MF). Following the pre-experimental period, the twelve subjects were randomly assigned so that four subjects would receive one of three experimental diets for an 18-day diet period: a low linolenic canola oil diet (LLNA), a regular canola oil diet (CAN), or a canola and flax oil diet (FLAX). All subjects were then returned to the MF diet for further 18 days. This diet period served as a "washout" period to return blood parameters to baseline values. During the final 18 days of the study, two subjects from each of the diet treatments in the first 18-day period were randomly assigned to one of the other two experimental diets (Figure 3). Thus, two of the subjects who had been assigned to the LLNA diet were randomly assigned to the CAN diet while the other two subjects were assigned to the FLAX diet.

3.2 Experimental Protocol

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.

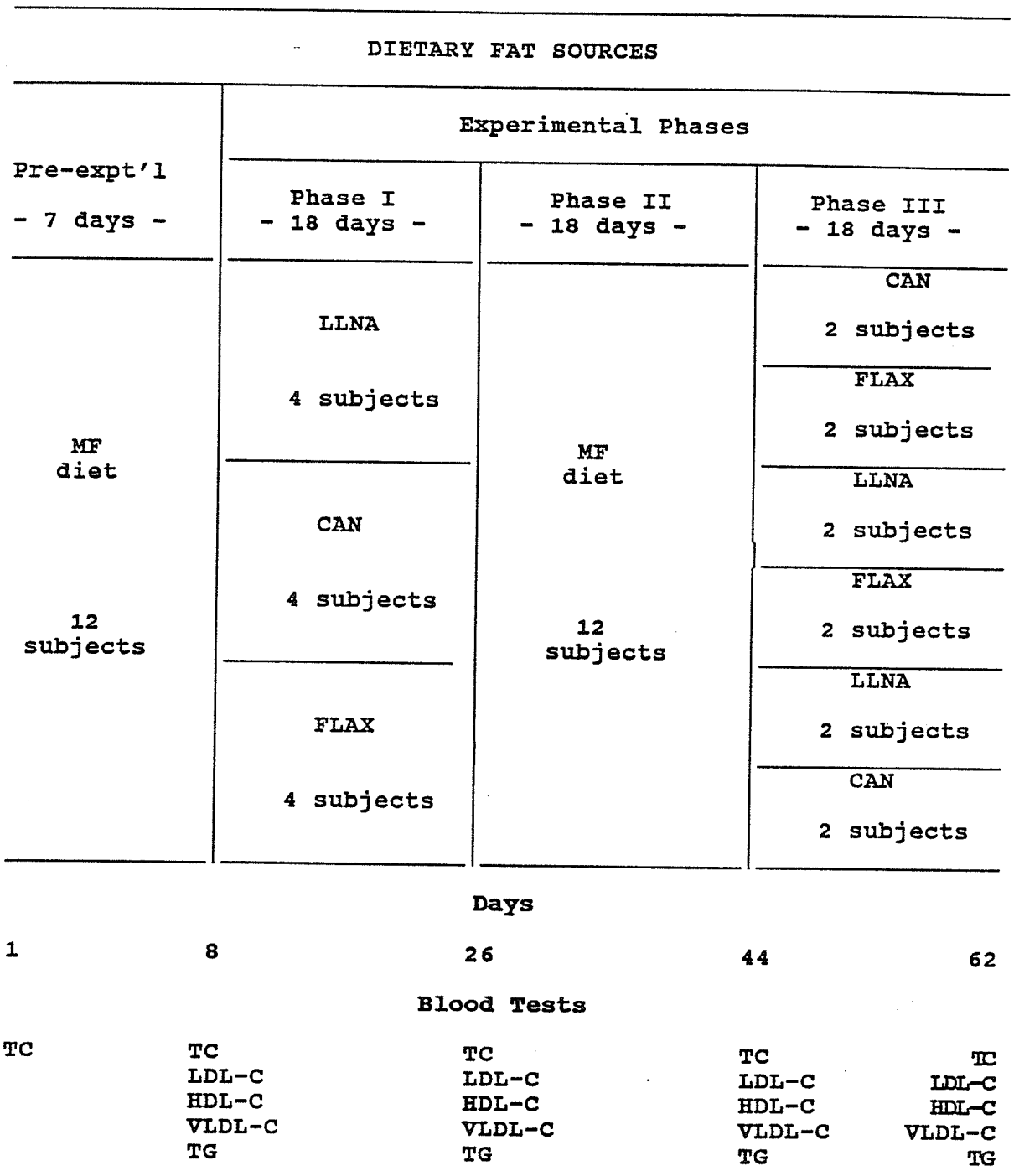


Figure 3. Experimental Design

Consumption of alcohol was prohibited. Subjects were advised not to take any medications without the consent of the study directors; in particular, the subjects were advised not to take aspirin or non-steroidal anti-inflammatory drugs (NSAID). The subjects were instructed to maintain their usual routines and customary activity patterns throughout the study.

3.3 Subjects

Subjects for the study were recruited by posting notices on campus and through an advertisement in the student newspaper. Potential subjects were interviewed and screened for plasma TC. The twelve male subjects chosen for the study were selected based on the absence of any family history of CHD, normolipidemic TC concentrations and the perception of their willingness to comply with the study protocol. In addition, the subjects were required to undergo a physical examination.

The physical data for the subjects is shown in Table 1. All subjects were students of the University of Manitoba and none of the subjects were taking any medications.

3.4 Diets

A two-day cyclic menu of conventional foods is shown in Table 2. The four diets, which were designed to be nutritionally adequate, were identical except for differences in the sources of added dietary fat. The diets provided

Table 1. Physical Data of the Subjects at Screening and Baseline

Subject	Height (cm)	Initial[1] Weight (kg)	BMI[2] (kg/m^2)	Screening[3] Plasma Cholesterol (mmol/L)
1	177.0	93.9	30.0	4.02
2	170.2	62.7	22.0	4.44
3	181.6	111.4	32.4	5.26
4	182.9	77.7	23.1	4.39
5	176.0	78.0	25.4	3.86
6	182.9	70.0	20.7	4.94
7	175.3	88.9	28.0	5.25
8	176.5	60.0	19.1	4.26
9	185.4	80.2	22.5	4.47
10	175.3	60.5	19.8	3.91
11	175.3	71.6	23.1	4.48
12	166.4	63.6	23.2	5.01

[1] Measured on day 1.
[2] Body Mass Index.
[3] Measured two weeks prior to commencement of the study.

approximately 3120 kcalories/day, with 53% of energy provided by carbohydrate (CHO), 14% by protein and 33% by fat (Table 3).

The added dietary fat accounted for approximately 79% of the total dietary fat or 29% of total energy. The added fat in the MF diet consisted of a mixture of fats designed to provide the same level of SFA as the average Canadian diet. The sources of added fat for the different experimental diets are outlined in Table 4. The distribution of added fat among the various foods in the diets is shown in Table 5. The fatty acid composition of the oils is presented in Appendix 3. The experimental diets contained about one-third the amount of SFA present in the MF diet. The fatty acid composition of the experimental diets was manipulated so that the diets would contain similar amounts of LA but different amounts of LNA, which were varied at the expense of OA (i.e., the amount of LNA varied in the diets as well as the LA/LNA ratio). To achieve this, sunflower oil was added to the CAN and FLAX diets to provide the same amount of LA as the LLNA diet, and flax oil was added to the FLAX diet to provide a higher level of LNA than the LLNA and CAN diets. Hence, the ratio of LA/LNA for the experimental diets were: 6.3 for the LLNA diet; 4.3 for the CAN diet; and 2.5 for the FLAX diet (Table 6). The amount of cholesterol contributed by the fat sources in the MF diet was approximately 100 mg higher than in the experimental diets due to the presence of animal fat.

Table 2. Two-day Cyclic Menu[1]

	Food Item (Amount)	
	Day I	Day II
Breakfast	Orange juice (125 ml) Granola (40 g) Skim milk (125 ml)	Apple juice (125 ml) Granola (40 g) Skim milk (125 ml)
Lunch	Chili (75 g) Lettuce (30 g) Oil (4 g) & vinegar (4 g) Canned Pears (125 g) Skim milk (250 ml)	Chicken casserole (75 g) Noodles (25 g) Celery (25 g) Raw Carrots (50 g) Canned Pineapple (125 g)
Dinner	Sliced Chicken (75 g) Rice (40 g) Kernel Corn (125 g) Jellied fruit (125 g) Skim milk (250 ml)	Hamburger Patty (75 g) Scalloped Potatoes (75g) Peas (125 g) Canned Peaches (125 g) Skim milk (250 ml)
Snacks	Raw apple (1 medium) Cookies (3) Skim milk (250 ml)	Raw apple (1 medium) Cookies (3) Skim milk (250 ml)
Bread	7 slices	7 slices
Jam or Diet Jam	2 packages (28.4 g)	2 packages (28.4 g)
Spread	1 package (40 g)	1 package (40 g)

[1] Coffee, tea, diet soft drinks and artificial sweetner 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 4 for recipes.
[4] Skinless boneless chicken breast.
[5] Top round ground beef.

Table 3. Macronutrient Content of the Diets[1]

NUTRIENT	WEIGHT (g)	% OF TOTAL ENERGY[2]
CHO	411	53
PRO	108	14
FAT	116	33

[1] Means of four two day duplicate diet samples.
[2] Total energy of the diets was 3120 kcalories.

Table 4. Sources of Added Dietary Fat[1]

DIET	FAT SOURCE
MIXED FAT[2]	10% Corn oil 20% Lard 20% Tallow 20% Crisco Shortening 30% Butter
LLNA[3]	100% Low Linolenic Canola oil
CAN[4]	85% Canola oil 15% Sunflower oil
FLAX[5]	67% Canola oil 18% Sunflower oil 15% Flax oil

[1] Accounted for approximately 79% of total dietary fat. The remaining 21% was contributed by invisible fat present in foods used in the diet.

[2] Mazola Corn oil, Best Foods Division, Canada Starch Co. Ltd., Montreal, PQ; Tenderflake Lard, Canada Packers Ltd., Toronto, ON; Tallow, Canada Packers Ltd., Winnipeg, MB; Crisco Shortening, Procter and Gamble, Toronto, ON; Inwood Butter, The Inwood Creamery Co., Inwood, MB.

[3] Low Linolenic Canola oil supplied courtesy of CSP Foods, Winnipeg, MB.

[4] Canola oil supplied courtesy of CSP Foods, Winnipeg, MB; Sunflower oil supplied courtesy of CSP Foods, Winnipeg, MB.

[5] Flax oil, Omega Nutrition Inc., Vancouver, BC.

Table 5. Daily Distribution of Added Fat in the Diets

Food Item	Diet			
	MF[1] (g)	LLNA (g)	CAN (g)	FLAX[2] (g)
DAY 1				
Granola	12	12	12	12
Chili	21	21	21	21
Salad Dressing	4	4	4	4
Rice	13	13	13	13
Kernel Corn	6	6	6	6
Cookies	18	18	18	18
Spread	32	32	32	32
DAY 2				
Granola	12	12	12	12
Chicken Casserole	15	15	15	15
Noodles	4	4	4	4
Scalloped Potatoes	25	25	25	25
Cookies	18	18	18	18
Spread[3]	32	32	32	32

[1] 4 g/day of corn oil consumed as salad oil and 40 g/day of butter (32 g/day of butter fat) was consumed as spread. Lard, tallow, shortening (21 g/day each) and 6.3 g of corn oil were blended to a uniform consisitency and used in baking and preparing the other food items.

[2] 16 g/day of flax oil was added to chili or scalloped potatoes prior to serving. 19 g/day of sunflower oil was mixed with 71 g/day of canola oil and used in baking and preparing the other food items.

[3] Spreads for the experimental diets were made by blending sunflower and canola oils with butter; 6 g/day butter (4.8 g of butter fat) and 26 g of oil mixture were used.

Table 6. Levels of Selected Fatty Acids in the Diets

Fatty Acid (% of total)	DIET[1]			
	MF	LLNA	CAN	FLAX
SFA	43.0	14.0	14.0	15.0
OA	39.0	53.0	51.0	45.0
LA	12.0	25.0	26.0	27.0
LNA	1.0	4.0	6.0	11.0
LA/LNA	12.0	6.3	4.3	2.5

[1] Means of two duplicate diet samples.

Therefore, to maintain a constant dietary cholesterol intake, egg yolk was added to various recipes in the experimental diets.

All meals except breakfast were served in the metabolic unit in the Faculty of Human Ecology at the University of Manitoba. Breakfast meals were packed for consumption at home. Meals were served at customary times but individual schedules were accommodated as necessary. All food and beverages, including coffee, tea, artificial sweetner and diet soft drinks, were provided throughout the study. Emphasis was placed on the fact that no foods other than those provided were to be eaten.

The subjects weighed themselves daily at approximately the same time each day. Energy intake was adjusted to maintain constant body weight (within ±1 kg) throughout the study. When adjustments were necessary, careful attention was taken to maintain the calorie contribution provided by fat (33% of total energy) and the other macronutrients.

3.5 Handling, Preparation and Storage of Food Staples

All food was purchased as single lots from local suppliers, except for fresh produce, skim milk and bread, which were purchased bi-weekly from a local retail grocer. The fresh produce and skim milk were stored at 7°C in a conventional refrigerator, whereas the bread was stored at -10°C until needed. Dry staples, canned fruits, and frozen

juice concentrate and frozen vegetables were stored under appropriate conditions. All fats and experimental oils were refrigerated except for the flaxseed oil which was stored at -10°C.

Diet items, such as cookies and granola, were prepared from standardized recipes (Appendix 4) and frozen prior to the beginning of the study. Ground beef for chili, and skinless, boneless chicken breasts were cooked, portioned, and frozen. Hamburger patties were portioned raw and frozen. All portions were stored at -10°C in a walk-in freezer. Spread was blended just prior to the beginning of the study and stored at -10°C until required. All remaining menu items were prepared in the metabolic unit prior to serving. All items were prepared according to the standardized recipes by weighing on a balance (Mettler PM4000). The menu items, recipes, and storing and handling procedures were similar to those utilized in previous studies (Corner, 1989; Chan, 1990; Chard, 1991).

3.6 Diet Analysis

All food items in the 2-day cyclic menu for each of the diets were combined, homogenized in a Waring blender, and aliquots were taken for proximate analysis. The aliquots were lyophilized to determine the moisture content of the samples. The protein content of the diets was determined in the Department of Animal Science by the Kjeldahl method. The fat content was determined by the method of Bligh and Dyer (1959).

The CHO content was derived by difference. The fatty acid composition of the extracted fat was determined by gas chromatography (GC), following methylation of the fatty acids using sodium methoxide in methanol (Bannon et al., 1985). The fatty acid methyl esters were analyzed with a Hewlett Packard 5890 Gas Chromatograph equiped with a Durabond-225 capillary column, 30 m x 0.25 mm, film thickness 0.25 microns (J & W Scientific Inc.). Injector, detector, and column temperatures were 250°C, 250°C, and 205°C, respectively. The carrier gas was helium. Peak areas were measured with a Hewlett Packard 3392A integrator. The settings for the gas chromatograph are presented in Appendix 5. Fatty acid peaks were identified using reference samples (cat. # GLC-68B, and GLC-87, Nu-Chek Prep. Inc., Elysian, MN). Sample chromatograms of the references GLC-68B and of a diet are included in Appendices 6 and 7, respectively.

3.7 Plasma Lipid and Lipoprotein Cholesterol Analysis

Venous blood samples were taken from each subject following a 12-hour overnight fast on Days 1, 8, 20, 26, 38, 44, 56 and 62. Blood was taken from the antecubital vein using vacutainer tubes containing 0.10 mL of 15% EDTA (K_3) solution as anticoagulant. Plasma lipid and LP analyses were performed on fresh samples within three days of collection. LP fractions in platelet-poor plasma (PPP; see below for preparation of PPP) were separated by ultracentrifugation

(Lindgren, 1975). The HDL fraction and VLDL fraction were separated by centrifuging 0.6 mL plasma samples at densities of 1.063 g/mL and 1.0063 g/mL, respectively, for 18 hours at 104,000 x g by the method of Bronzert and Brewer (1977). Cholesterol in the plasma and LP fractions was determined enzymatically by the method of Allain et al. (1974) using a diagnostic kit (Diagnostic Chemicals Limited, Charlottetown, PEI). Plasma TG were analysed using a diagnostic kit (Diagnostic Chemicals Limited, Charlottetown, PEI) according to the enzymatic method by Fossatti and Lorenzo (1982) as modified by McGowan et al. (1983).

3.8 Platelet Phospholipid Fatty Acid Analysis

Platelets were isolated from blood samples on days 8, 26, 44 and 62. Red blood cells were removed from the blood by centrifuging at 250 x g for 15 minutes at 18°C. The platelets were separated by centrifuging the plasma at approximately 1400 x g for 10 minutes at 4°C as described by McKean et al. (1981). The resulting platelet-poor plasma (PPP) was decanted from the samples and used for the plasma lipid and LP analyses. The platelet pellets were washed with buffered saline containing EDTA, re-centrifuged 10 minutes at 1400 x g at 4°C, re-suspended with buffered saline without EDTA but containing 5mM D-glucose, flushed with nitrogen, and stored at -20°C until required for use in the platelet PL fatty acid analysis.

Lipids were extracted from the platelets by the method of Bligh and Dyer (1959). PL fractions of the platelets were separated using two-dimensional thin-layer chromatography (TLC) essentially as described by Thomas and Holub (1988). Each sample was spotted under nitrogen onto a heat-activated, pre-coated, 20 x 20 cm^2 thin layer plates (silica gel 60, Merck & Co.; 0.25 mm thick) using a 5 ul SGE syringe. Plates were developed for 95 minutes in the first direction using a solvent system consisting of chloroform/methanol/ammonium hydroxide (65/35/5.5; v/v/v). Each plate was dried under nitrogen for 30 minutes and exposed to hydrochloric acid fumes for 10 minutes to hydrolyse the ether linkages in the PPE, thus enabling the separation of PE from 2-acyl-ethanolamine phosphoglyceride. The plates were dried for another 30 minutes and then developed in the second direction for 110 minutes using the solvent system chloroform/methanol/formic acid (55/25/5; v/v/v). The developed plates were dipped in a dichlorofluorescein solution, exposed to ammonia fumes for 10 minutes and observed under UV light. The different PL spots were identified using a standard plate supplied by the Department of Nutritional Sciences, University of Guelph (Thomas and Holub, 1988). PC, PE and PPE spots were scraped from the plate and transferred to screw-cap tubes for methylation. Methylation and analyses of the fatty acid composition was carried out as described earlier under "Diet Analysis" except that the GC setting differed slightly from

that used for the diet analyses (see Appendix 5). Fatty acid peaks were identified by comparison to standard chromatograms as described earlier but also containing peaks for 20:5, 22:3 and 22:4 (Nu-Chek Standards U-100-M, U-82-M, and U-83-M, resp.). Pentadecaenoic acid (15:0) was used as an internal standard in all samples. Sample chromatograms of the standards and each of the PL fractions are included in Appendices 6 (A-E) and 8-10, respectively.

Methylation blanks were prepared each time a set of samples were methylated. In addition, a blank spot was scraped from each TLC plate, methylated and analyzed by GC as described for the PL spots. The fatty acid composition of the PL fractions were corrected by subtracting the amounts of the fatty acids in the blank spots from the amounts of these fatty acids in the PL fractions, relative to the amount of 15:0 (internal standard) in each PL fraction.

3.9 Statistical Analysis

The plasma lipid and LP data and the platelet PL fatty acid data were analyzed using a combined intra- and inter-block analysis designed for a BIBD. A two-way ANOVA model in the PROC GLM of SAS was used for the analyses (SAS Institute Inc., Cary, NC 1984, 1986). Pairwise comparisons were made between the LLNA and FLAX diets and the LLNA and CAN diets for both plasma lipids and LP, and platelet PL data. Combined anlayses indicated that block-to-block (subject-to-subject)

variability in the model was not significant in both the plasma lipid and LP and platelet PL analyses. t-Test contrasts were used to determine the significance of the experimental diet effects relative to the effects of the MF diet and also to compare the significance of the diet effects among the experimental diets. t-Test contrasts were also employed to determine the effect of the washout period. Thus, plasma and LP cholesterol levels and platelet PL fatty acid levels following the washout phase (day 44) were compared to levels following the pre-experimental phase (day 8). The associated p-values accompanied all t-test analyses.

4. RESULTS

4.1 Subjects

All subjects completed the 61-day study and remained highly motivated throughout the study. Subject compliance to the dietary regimen, which was monitored by weight records and personal contact with the subjects, was considered satisfactory. In general, the subjects' weights did not change more than ±1 kg, except for four subjects: subject 3 lost 3.6 kg, subject 9 lost 4.5 kg, subject 7 lost 3.2 kg, and subject 10 gained 3.2 kg. The weight changes in these subjects occurred slowly and steadily over the duration of the study. Subject weight loss was not due to caloric restriction but was attributed to improvements in the meal eating patterns of subjects 3 and 7 and to increased physical activity for subject 9. The weight gain in subject 10 occurred towards the end of the study and could not be adequately explained.

4.2 Plasma Total and Lipoprotein Cholesterol Levels

Changes in plasma TC and LP cholesterol levels in response to the experimental diets are reported as the mean change in plasma cholesterol concentration (mmol/L) following each of the experimental diets compared to the MF diet (Tables 7 & 8).

4.2.1 Effect of the Experimental Diets (Tables 7 & 8)

Plasma TC levels decreased considerably in response to the experimental diets. The decrease in TC levels were mainly due to a decrease in LDL-C levels. TC levels were on average -0.80 mmol/L lower (-18%) while LDL-C levels were -0.60 mmol/L lower (-21%) following the experimental diets. The decrease in plasma TG was statistically significant ($p<0.05$) only for the FLAX diet (-0.18 mmol/L; -17%) whereas the decrease in VLDL-C levels was statistically significant ($p<0.01$) only for the CAN diet (-0.18 mmol/L; -70%). The experimental diets did not affect HDL-C levels except for the small significant ($p<0.05$) decrease of -0.11 mmol/L (-9%) on the FLAX diet. The reductions in plasma lipid and LP levels are shown in Figures 4-8.

There were no significant differences in the changes in plasma lipid and LP levels among the experimental diets (Tables 7 & 8). Thus, changes in plasma lipids followed a similar pattern relative to the MF diet regardless of the experimental diet. In some cases, the changes were statistically significant for all diets (viz., TC and LDL-C), whereas in other cases the magnitude of change only reached statistical significance on one diet (e.g., decreases in TG and HDL-C on the FLAX diet and VLDL-C on the CAN diet). Mean plasma lipid and LP levels on the various diets are shown in Table 9.

Table 7. Mean Changes in Plasma Total and Lipoprotein Cholesterol Levels on the Experimental Diets

Plasma Lipid (mmol/L)	Experimental Diets		
	LLNA	CAN	FLAX
TC	-0.66±0.10[†a]	-0.73±0.10[†a]	-0.94±0.10[†a]
LDL-C	-0.60±0.09[†a]	-0.52±0.09[†a]	-0.71±0.09[†a]
HDL-C	0.02±0.04[a]	-0.04±0.04[a]	-0.11±0.04[†a]
VLDL-C	-0.06±0.06[a]	-0.18±0.06[†a]	-0.11±0.06[a]
TG	-0.14±0.08[a]	-0.13±0.08[a]	-0.18±0.08[†a]

[1] All values are mean ± SE.
[2] Means in the same row with the same superscript letter do not differ ($p>0.05$).
[†] [3] Values differ significantly from the MF diet ($p>0.05$).
Plasma total and lipoprotein cholesterol data of individual subjects is shown in Appendix 13. p-Values for the statistical comparison of each experimental diet to the MF diet are shown in Appendix 17 and p-values for the statistical comparison among the experimental diets are shown in Appendix 18.

Table 8. Percent Changes in Plasma Total and Lipoprotein Cholesterol Levels on the Experimental Diets

PLASMA LIPID (mmol/L)	EXPERIMENTAL DIET		
	LLNA	CAN	FLAX
TC	-15%†	-17%†	-21%†
LDL-C	-20%†	-18%†	-23%†
HDL-C	1%	-2%	-9%†
VLDL-C	-23%**	-70%†	-41%*
TG	-6%	-13%	-17%†

† Significantly different ($p<0.05$) from the MF diet.
* Mean decrease in VLDL-C levels in 7 subjects.
** Mean decrease in VLDL-C levels in 6 subjects.

Table 9. Mean Plasma Total and Lipoprotein Cholesterol Levels on the Mixed Fat and Experimental Diets[1]

Plasma Lipid (mmol/L)	DIETS			
	MF n=24	LLNA n=8	CAN n=8	FLAX n=8
TC	4.44±0.10[2]	3.72±0.18	3.59±0.12	3.68±0.21
LDL-C	3.06±0.09	2.42±0.18	2.43±0.13	2.49±0.21
HDL-C	1.13±0.04	1.13±0.09	1.10±0.06	1.03±0.07
VLDL-C	0.25±0.04	0.17±0.06	0.06±0.02	0.17±0.07
TG	0.91±0.08	0.84±0.14	0.64±0.06	0.81±0.11

[1] A table of the mean values on each of the diets. Conclusions were not based on these values but on the changes that occurred when subjects were changed from the MF to the experimental diets. Refer to Tables 7 and 8 for the statistical analysis of the diet effects.

[2] All values are mean ± SE.

Figures 4-8. Changes in Plasma Lipid and Lipoprotein Cholesterol Levels on the Experimental Diets.

Figure 4. Changes in Total Cholesterol Levels on the Experimental Diets

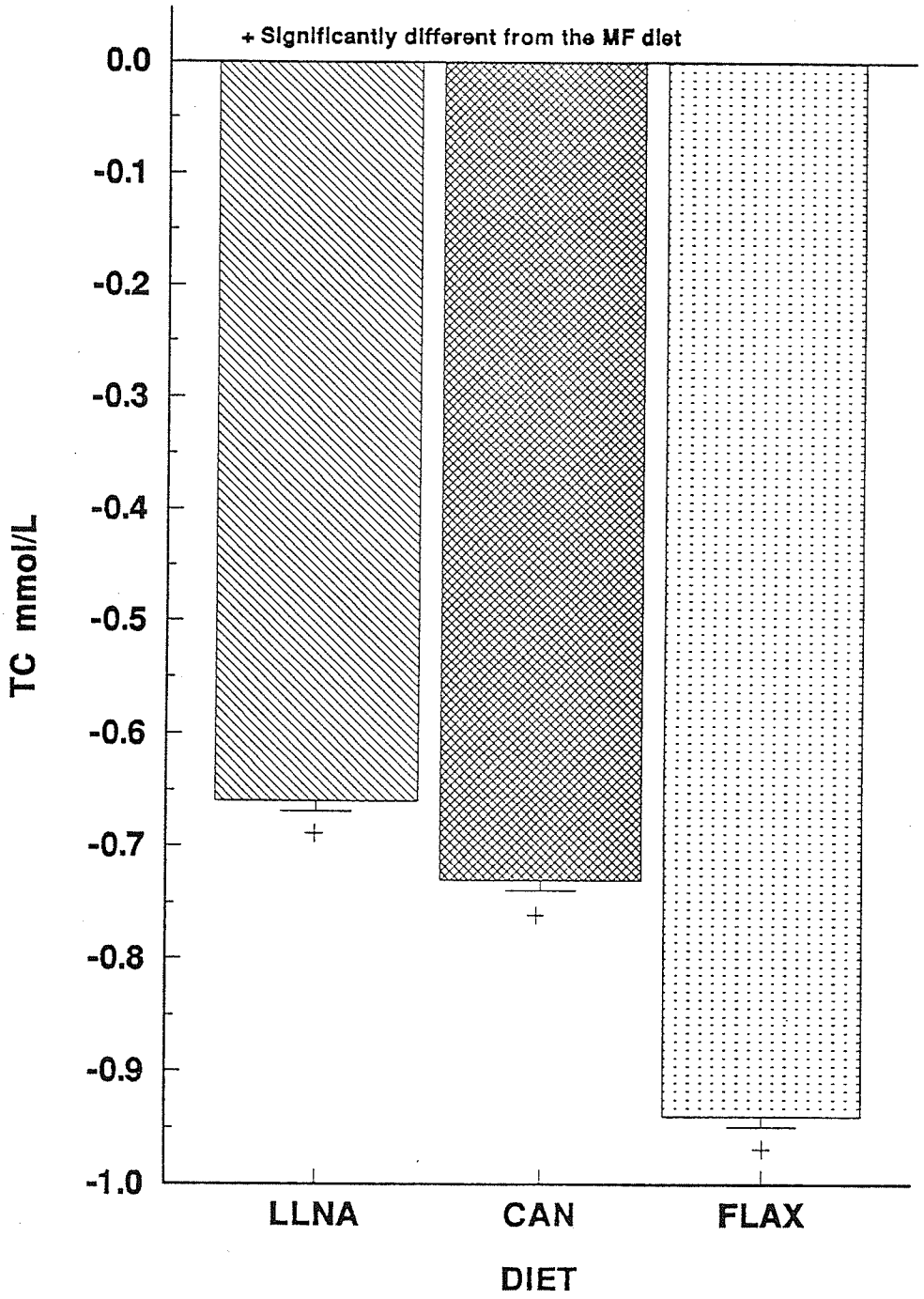


Figure 5. Changes in LDL-C Levels on the Experimentl Diets

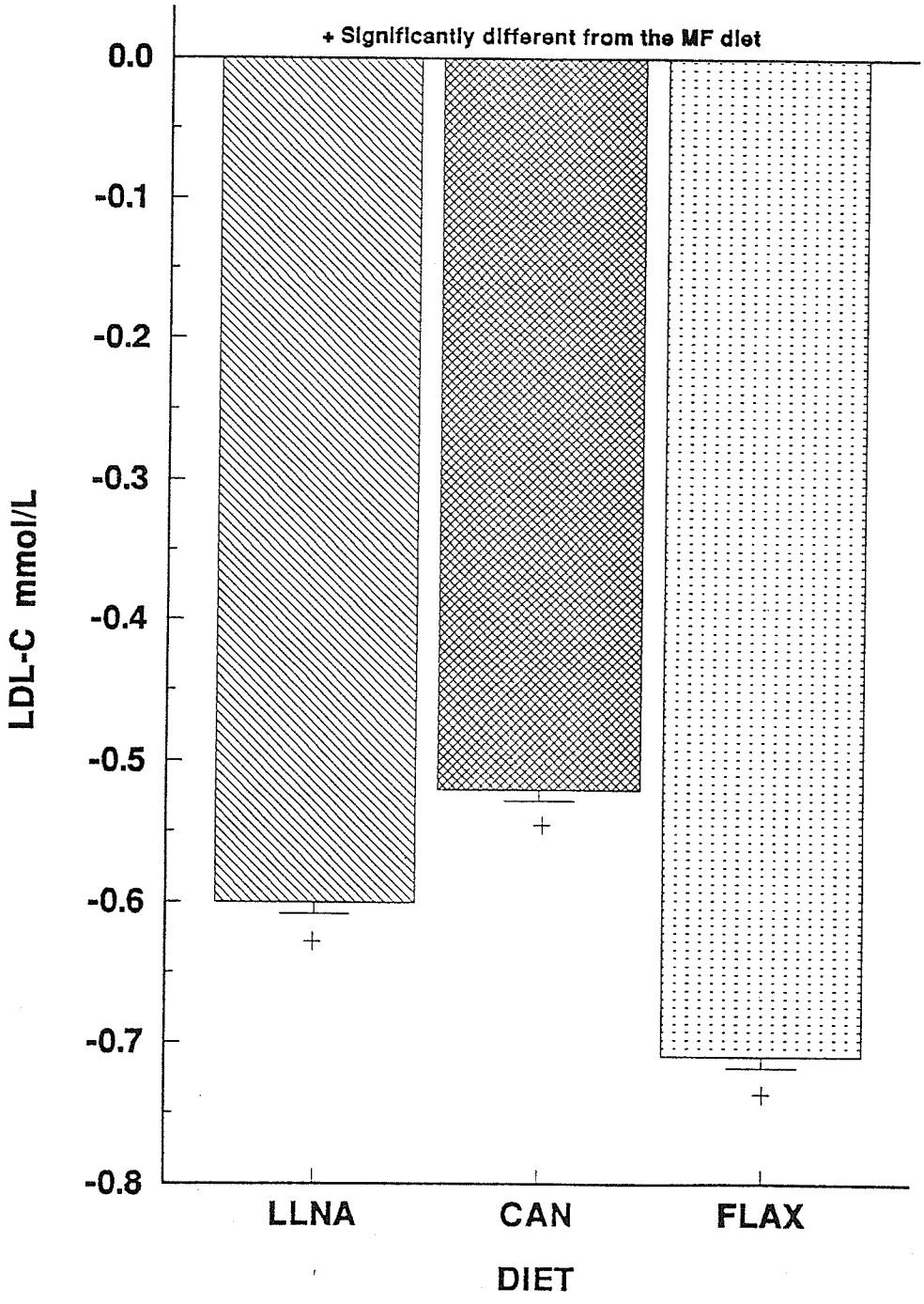


Figure 6. Changes in HDL-C Levels on the Experimental Diets

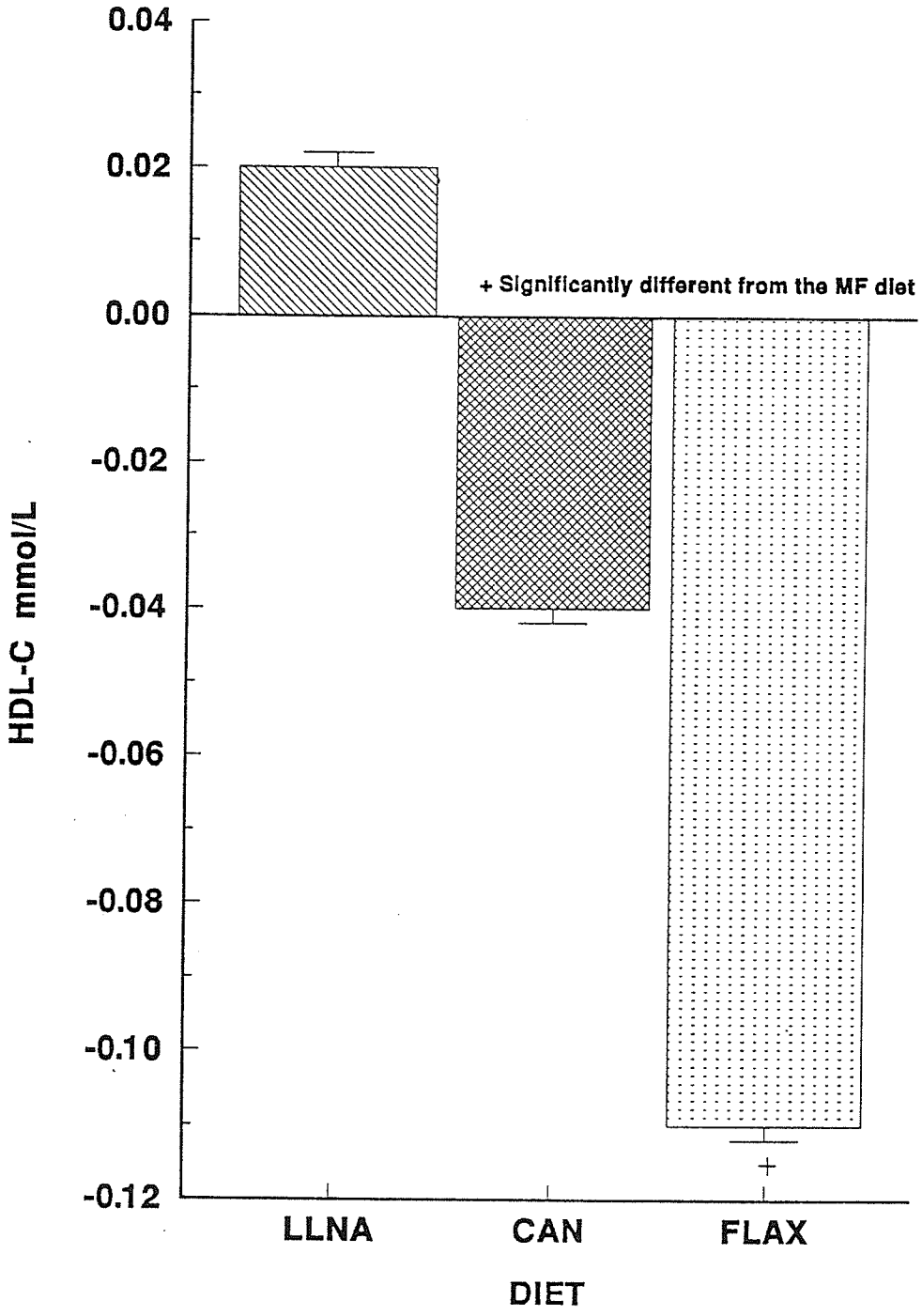


Figure 7. Changes in VLDL-C Levels on the Experimental Diets

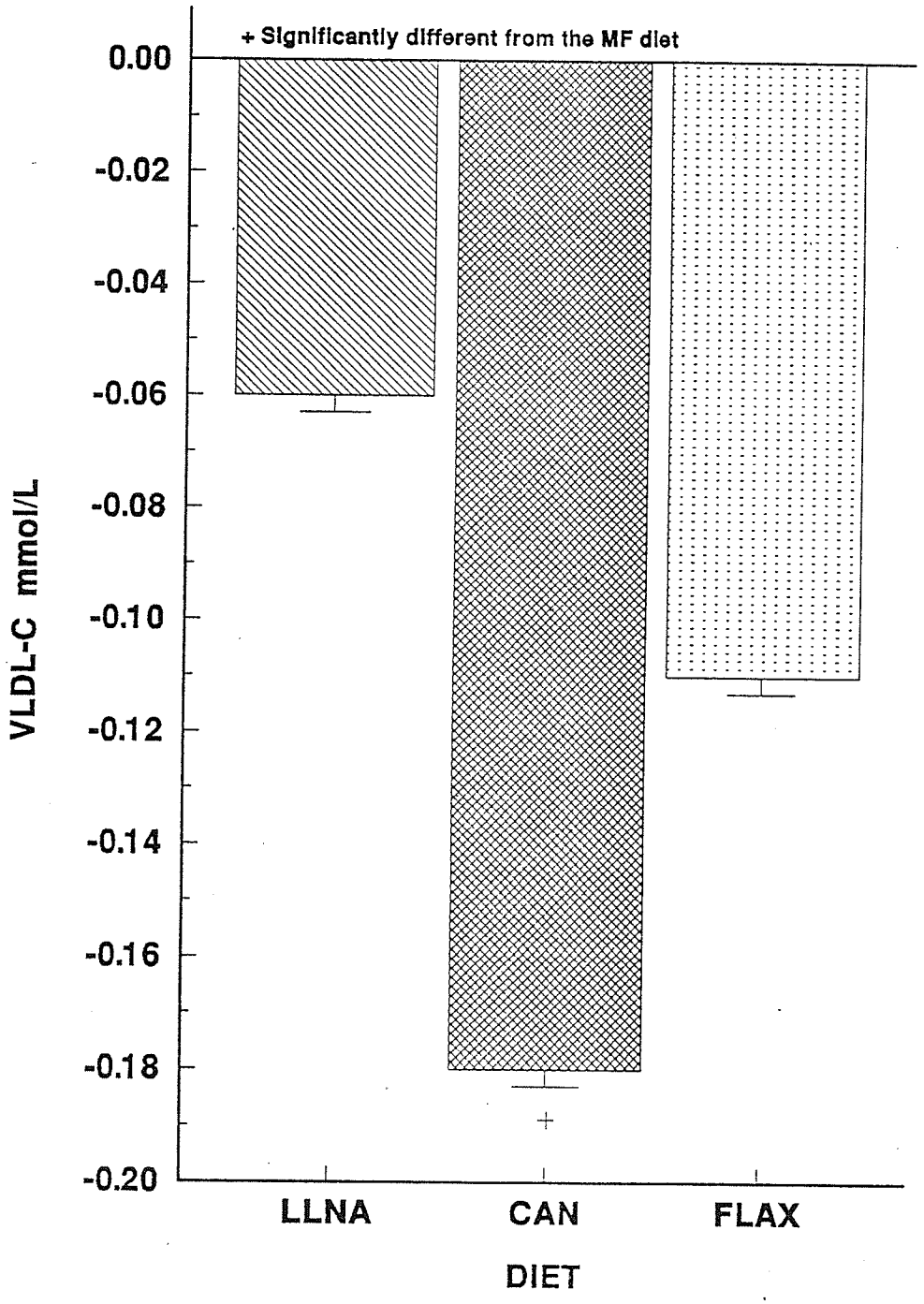
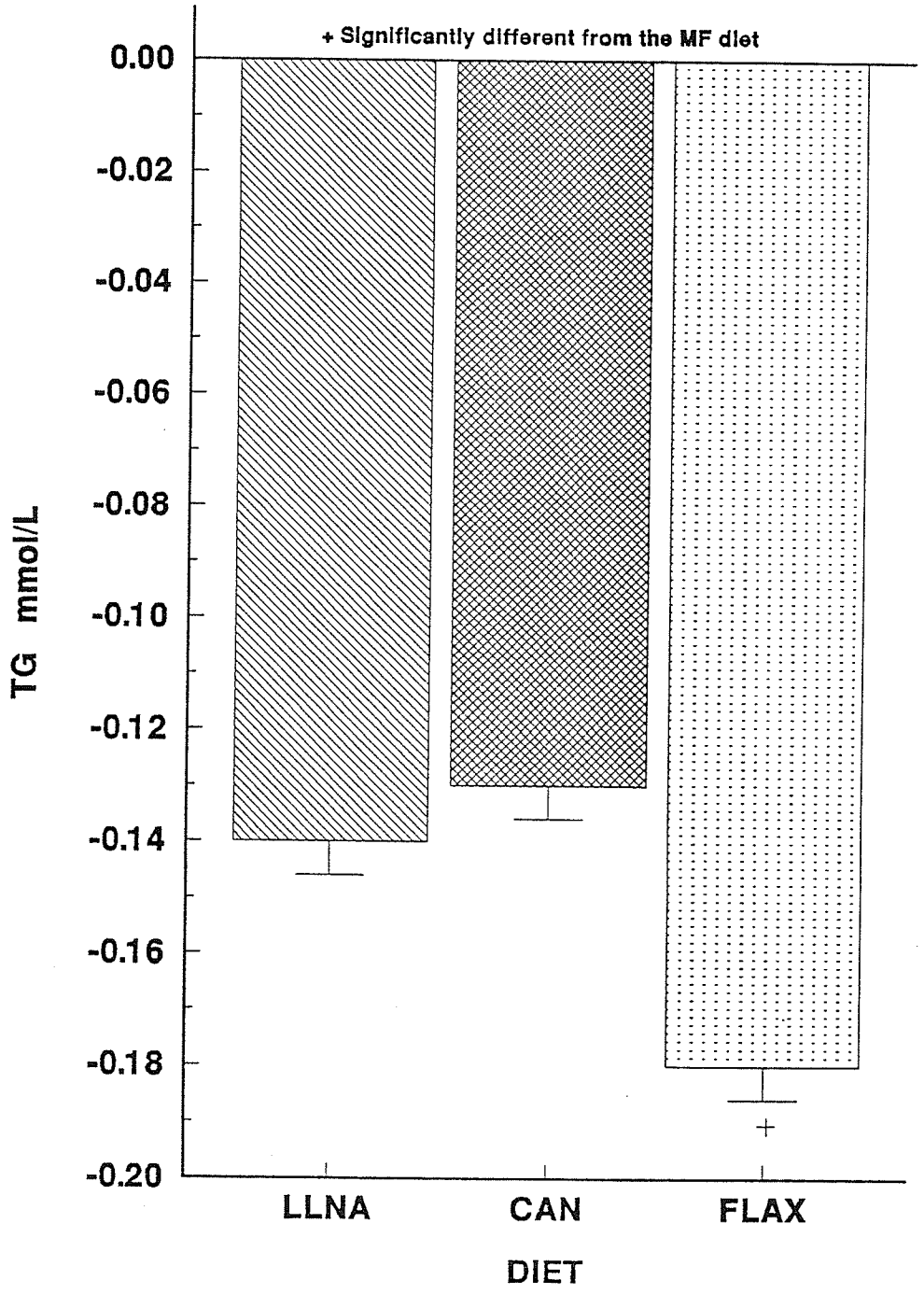


Figure 8. Changes in Triglyceride Levels on the Experimental Diets



4.2.2 Effect of the Washout Phase on Plasma Total and Lipoprotein Cholesterol Levels (Table 10)

TC, LDL-C, HDL-C and VLDL-C levels returned to pre-experimental levels (day 8; $p<0.05$) when the subjects consumed the MF diet for the 18-day washout phase of the study (day 44; Table 10). However, plasma TG levels were higher ($p<0.01$) following the washout phase compared to levels following the pre-experimental period.

4.3 Platelet Phospholipid Fatty Acid Patterns

The platelet PC, PE and PPE fatty acid data were subjected to the same statistical analyses as the plasma lipid and LP data. Totals for the PUFA of the n-3 and n-6 families with chain lengths greater than 18 carbons (LC PUFA) were calculated in an attempt to determine whether the ratio of dietary LA/LNA had any apparent effect on the elongation-desaturation pathway for the 18-carbon isomers of these fatty acids.

4.3.1 Effect of the Experimental Diets

Differences between the MF diet and the experimental diets were statistically analyzed for mean differences between values on day 8 versus day 26 and the mean difference of values on day 44 versus day 62.

Table 10. Mean Differences in Plasma Total and Lipoprotein Cholesterol Levels Following the Washout and Pre-Experimental Phases (Day 44 vs. Day 8)

Plasma Lipid	Mean Difference (mmol/L)	Probability (p>t)
TC	-0.03±0.05[1]	0.82
LDL-C	-0.02±0.07	0.73
HDL-C	-0.04±0.05	0.47
VLDL-C	0.03±0.06	0.56
TG	0.16±0.05†	0.01

[1] All values are means ± SE.
† Values are significantly different ($p<0.05$) from values following pre-experimental period (day 8).

4.3.1.1 Phosphatidlycholine (PC) (Table 11)

All of the experimental diets resulted in a decrease in the levels of STEA, ETA, AA and the total n-6 LC PUFA and an increase in the levels of LA, LNA and EDA of platelet PC compared to levels on the MF diet. There was no changes in the level of EPA on the LLNA and CAN diets whereas there was an increase ($p<0.02$) in the EPA level on the FLAX diet. Levels of DPA and total n-3 LC PUFA decreased on the LLNA and CAN diets but there was no change in these levels on the FLAX diet. However, the level of DTA decreased on the CAN diet but not on the FLAX and LLNA diets. The CAN diet also resulted in a decrease ($p<0.01$) in the level of DHA while the other diets did not affect DHA levels. On the other hand, none of the experimental diets had any effect on the levels of PMA or OA, even though the diets differed in OA content (Table 6).

4.3.1.2 Phosphatidylethanolamine (PE) (Table 12)

The experimental diets resulted in a decrease in the levels of STEA, AA and total n-6 LC PUFA and an increase in the levels of OA, LA and LNA in platelet PE. Levels of EDA increased on the LLNA diet but were unchanged on the CAN and FLAX diets, whereas levels of ETA increased in response to the LLNA and CAN diets but remained unchanged in response to the FLAX diet. There was a decrease in the level of DPA on the LLNA and CAN diets but no change on the FLAX diet. Levels of EPA and total n-3 LC PUFA decreased on the LLNA diet but did

Table 11. Mean Changes in Platelet Phosphatidylcholine Fatty Acid Levels on the Experimental Diets

Fatty Acid[2]	Experimental Diets		
	LLNA	CAN	FLAX
16:0	0.63±0.81[1]	0.60±0.81	0.28±0.81
18:0	-2.13±0.28†	-1.90±0.28†	-1.74±0.28†
18:1	0.72±0.50	0.38±0.50	0.12±0.50
18:2	1.59±0.25†	1.74±0.25†	1.85±0.25†
18:3	0.05±0.01†	0.13±0.01†	0.22±0.01†
20:2	0.20±0.02†	0.19±0.02†	0.22±0.02†
20:3	-0.30±0.05†	-0.36±0.05†	-0.39±0.05†
20:4	-1.79±0.45†	-2.22±0.45†	-1.80±0.45†
20:5	-0.03±0.02	-0.03±0.02	0.05±0.02†
22:4	-0.09±0.07	-0.19±0.07†	-0.13±0.07
22:5	-0.15±0.04†	-0.13±0.04†	0.03±0.04
22:6	-0.06±0.05	-0.15±0.05†	-0.02±0.05
n-3 PUFA[3]	-0.22±0.09†	-0.32±0.09†	0.06±0.09
n-6 PUFA[4]	-2.16±0.49†	-2.75±0.49†	-2.35±0.49†

[1] All values are means ± SE and expressed as a % of total fatty acids in the PC fraction. Platelet PC data for individual subjects are shown in Appendix 14. A sample fatty acid chromatogram for platelet PC is shown in Appendix 8.

[2] Carbon chain length:number of double bonds.

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

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

† Values are significantly different ($p<0.05$) from levels on the MF diet. p-Values for the statistical comparison of each experimental diet to the MF diet are shown in Appendix 19.

Table 12. Mean Changes in Platelet Phosphatidylethanolamine Fatty Acid Levels on the Experimental Diets

Fatty Acid[2]	Experimental Diets LLNA	CAN	FLAX
16:0	-0.71±0.40[1]	-1.07±0.40†	-0.62±0.40
18:0	-3.26±0.72†	-2.90±0.72†	-2.41±0.72†
18:1	7.18±0.61†	5.59±0.61†	5.02±0.61†
18:2	1.34±0.12†	1.29±0.12†	1.90±0.12†
18:3	0.06±0.02†	0.08±0.02†	0.17±0.02†
20:2	0.42±0.13†	0.06±0.13	0.23±0.13
20:3	0.16±0.07†	0.22±0.07†	0.10±0.07
20:4	-5.53±0.78†	-3.78±0.78†	-4.73±0.78†
20:5	-0.04±0.02†	-0.01±0.02	0.08±0.02†
22:4	-0.22±0.19	-0.19±0.19	-0.57±0.19†
22:5	-0.49±0.08†	-0.23±0.08†	0.06±0.08
22:6	-0.17±0.09	-0.11±0.09	-0.05±0.09
n-3 PUFA[3]	-0.70±0.17†	-0.36±0.17	0.06±0.17
n-6 PUFA[4]	-5.49±0.90†	-3.77±0.90†	-5.29±0.90†

[1] All values are means ± SE and expressed as a % of total fatty acids in the PE fraction. Platelet PE data for individual subjects are shown in Appendix 15. A sample fatty acid chromatogram for platelet PE is shown in Appendix 9.

[2] Carbon chain length:number of double bonds.

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

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

† Values are significantly different ($p<0.05$) from levels on the MF diet. p-Values for the statistical comparison of each experimental diet to the MF diet are shown in Appendix 20.

not change on the CAN diet. However, the level of EPA increased on the FLAX diet while the levels of total n-3 LC PUFA did not change. The CAN diet was associated with a decrease in the level of PMA while the FLAX diet was associated with a decrease in the level of DTA. The experimental diets did not affect levels of DHA in platelet PE.

4.3.1.3 Alkenylacyl Ethanolamine Phosphoglyceride (PPE) (Table 13)

There was an increase in the level of OA on all of the experimental diets in the platelet PPE fraction. Levels of PMA decreased on the FLAX diet but were unchanged on the CAN and LLNA diets. By contrast, STEA levels decreased on the LLNA and CAN diets but not on the FLAX diet. The LLNA and FLAX diets, but not the CAN diet, were associated with an increase in the content of LA. There was an increase in the level of EPA ($p<0.00004$) on the FLAX diet, whereas there was no changes in the EPA level on the LLNA and CAN diets. Levels of DPA and n-3 LC PUFA decreased in response to the LLNA diet, whereas there were no changes in these levels on the CAN and FLAX diets. There were no changes in the levels of LNA, ETA, AA, DTA, DHA and n-6 LC PUFA in response to the experimental diets, although increases in the levels of LNA and DPA approached statistical significance on the FLAX diet ($p<0.08$ and $p<0.06$, resp.).

Table 13. Mean Changes in Platelet Alkenylacyl Ethanolamine Phosphoglyceride Fatty Acid Levels on the Experimental Diets

Fatty Acid[2]	Experimental Diets LLNA	CAN	FLAX
16:0	-0.10±0.10[1]	-0.07±0.10	-0.27±0.10†
18:0	-0.47±0.18†	-0.57±0.18†	-0.29±0.18
18:1	0.93±0.28†	0.93±0.28†	0.98±0.28†
18:2	0.51±0.18†	0.37±0.18	0.80±0.18†
18:3	0.02±0.03	0.01±0.03	0.06±0.03
20:3	0.02±0.06	-0.01±0.06	-0.09±0.06
20:4	1.43±0.76	-0.19±0.76	0.32±0.76
20:5	-0.07±0.07	0.07±0.07	0.51±0.07†
22:4	-0.24±1.15	-0.07±1.15	-1.95±1.15
22:5	-1.53±0.42†	-0.47±0.42	0.89±0.42
22:6	-0.33±0.24	-0.41±0.24	-0.19±0.24
n-3 PUFA[3]	-1.92±0.66†	-0.82±0.66	1.21±0.66
n-6 PUFA[4]	1.51±1.15	-2.05±1.15	-1.84±1.15

[1] All values are means ± SE and expressed as a % of total fatty acids in the PPE fraction. Platelet PPE data for individual subjects are shown in Appendix 16. A sample fatty acid chromatogram for platelet PPE is shown in Appendix 10.

[2] Carbon chain length:number of double bonds.

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

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

† Values are significantly different ($p<0.05$) from levels on the MF diet. p-Values for the statistical comparison of each experimental diet to the MF diet are shown in Appendix 21.

4.3.2 Comparisons Among the Experimental Diets

Changes in fatty acid composition of platelet PL among the experimental diets also were analyzed statistically. Paired t-test contrasts were determined to compare the changes in platelet fatty acid content in response to the LLNA diet versus the FLAX diet and the LLNA diet versus the CAN diet. Comparisons between the effect of the FLAX diet and the CAN diet were made by deduction. Mean changes in fatty acid levels in platelet PL are shown in Tables 14-16 and mean fatty acid levels in platelet PL are shown in Tables 17-19.

4.3.2.1 Phosphatidylcholine (PC) (Table 14)

The experimental diets were associated with similar changes in the levels of PMA, STEA, OA, LA, EDA, ETA, AA, DTA, DHA and total n-6 LC PUFA in platelet PC. Changes in the levels of LNA reflected the LNA composition of the experimental diets. Hence, the magnitude of increase in LNA levels were least on the LLNA diet, intermediate on the CAN diet and greatest on the FLAX diet. Likewise, increases in the levels of EPA, DPA and total n-3 LC PUFA which occurred on the FLAX diet were different from the decreases in these levels on the LLNA and CAN diets.

4.3.2.2 Phosphatidylethanolamine (PE) (Table 15)

Changes in the levels of PMA, STEA, EDA, ETA, AA, EPA,

Table 14. Mean Changes in Platelet Phosphatidylcholine Fatty Acid Levels Among the Experimental Diets

Fatty Acid[2]	Experimental Diets LLNA	CAN	FLAX
16:0	0.63±0.81[13a]	0.60±0.81[a]	0.28±0.81[a]
18:0	-2.13±0.28[a]	-1.90±0.28[a]	-1.74±0.28[a]
18:1	0.72±0.50[a]	0.38±0.50[a]	0.12±0.50[a]
18:2	1.59±0.25[a]	1.74±0.25[a]	1.85±0.25[a]
18:3	0.05±0.01[a]	0.13±0.01[b]	0.22±0.01[c]
20:2	0.20±0.02[a]	0.19±0.02[a]	0.22±0.02[a]
20:3	-0.30±0.05[a]	-0.36±0.05[a]	-0.39±0.05[a]
20:4	-1.79±0.45[a]	-2.22±0.45[a]	-1.80±0.45[a]
20:5	-0.03±0.02[a]	-0.03±0.02[a]	0.05±0.02[b]
22:4	-0.09±0.07[a]	-0.19±0.07[a]	-0.13±0.07[a]
22:5	-0.15±0.04[a]	-0.13±0.04[a]	0.03±0.04[b]
22:6	-0.06±0.05[a]	-0.15±0.05[a]	-0.02±0.05[a]
n-3 PUFA[4]	-0.22±0.09[a]	-0.32±0.09[a]	0.06±0.09[b]
n-6 PUFA[5]	-2.16±0.49[a]	-2.75±0.49[a]	-2.35±0.49[a]

[1] All values are means ± SE and expressed as a % of total fatty acids in the PC fraction. Platelet PC data for individual subjects are shown in Appendix 14. A sample fatty acid chromatogram for platelet PC is shown in Appendix 8.

[2] Carbon chain length:number of double bonds.

[3] Values in rows with the same superscript letter do not differ ($p<0.05$). p-Values for the statistical comparison among the experimental diets are shown in Appendix 22.

[4] n-3 = 20:5 + 22:5 + 22:6.

[5] n-6 = 20:3 + 20:4 + 22:4.

Table 15. Mean Changes in Platelet Phosphatidylethanolamine Fatty Acid Levels Among the Experimental Diets

Fatty Acid[2]	Experimental Diets		
	LLNA	CAN	FLAX
16:0	-0.71±0.40[13a]	-1.07±0.40[a]	-0.62±0.40[a]
18:0	-3.26±0.72[a]	-2.90±0.72[a]	-2.41±0.72[a]
18:1	7.18±0.61[a]	5.59±0.61[a]	5.02±0.61[b]
18:2	1.34±0.12[a]	1.29±0.12[a]	1.90±0.12[b]
18:3	0.06±0.02[a]	0.08±0.02[a]	0.17±0.02[b]
20:2	0.42±0.13[a]	0.06±0.13[a]	0.23±0.13[a]
20:3	0.16±0.07[a]	0.22±0.07[a]	0.10±0.07[a]
20:4	-5.53±0.78[a]	-3.78±0.78[a]	-4.73±0.78[a]
20:5	-0.04±0.02[a]	-0.01±0.02[a]	0.08±0.02[a]
22:4	-0.22±0.19[a]	-0.19±0.19[a]	-0.57±0.19[a]
22:5	-0.49±0.08[a]	-0.23±0.08[b]	0.06±0.08[c]
22:6	-0.17±0.09[a]	-0.11±0.09[a]	-0.05±0.09[a]
n-3 PUFA[4]	-0.70±0.17[a]	-0.36±0.17[a]	0.06±0.17[b]
n-6 PUFA[5]	-5.49±0.90[a]	-3.77±0.90[b]	-5.29±0.90[a]

[1] All values are means ± SE and expressed as a % of total fatty acids in the PE fraction. Plasma PE data for individiual subjects are shown in Appendix 15. A sample fatty acid chromatogram for platelet PE is shown in Appendix 9.

[2] Carbon chain length:number of double bonds.

[3] Values in rows with the same superscript letter do not differ ($p<0.05$). p-Values for the statistical comparison among the experimental diets are shown in Appendix 23.

[4] n-3 = 20:5 + 22:5 + 22:6.

[5] n-6 = 20:3 + 20:4 + 22:4.

DTA and DHA in the platelet PE were similar on the experimental diets. Increases in the level of OA were greater as a result of the LLNA and CAN diets compared to the FLAX diet, which likely reflected the slightly higher content of OA in these diets. However, changes in the levels of LA and LNA were greater on the FLAX diet than on the LLNA and CAN diets. Changes in total n-3 LC PUFA levels on the FLAX diet also differed from those on the LLNA and CAN diets. In addition, changes in the content of DPA differed on the experimental diets. Decreases in total n-6 PUFA levels were less on the CAN diet compared to the LLNA and FLAX diets.

4.3.2.3 Alkenylacyl Ethanolamine Phosphoglyceride (PPE) (Table 16)

Changes in the levels of PMA, STEA, OA, LA, LNA, ETA, AA, DTA, DHA and total n-6 LC PUFA were similar among the experimental diets. However, the FLAX diet was associated with significantly different levels of EPA, DPA and n-3 LC PUFA from those on the LLNA and CAN diets.

4.3.3 Effect of the Washout Phase on Platelet Phospholipids (Tables 20, 21 & 22)

In general, the fatty acid composition of the platelet PL on day 44 returned to pre-experimental levels (day 8) when subjects were returned to the MF diet during the washout phase of the study (Tables 20-22). However, there were a few

Table 16. Mean Changes in Platelet Alkenylacyl Ethanolamine Phosphoglyceride Fatty Acid Levels Among the Experimental Diets

Fatty Acid[2]	Experimental Diets		
	LLNA	CAN	FLAX
16:0	-0.10±0.10[13a]	-0.07±0.10[a]	-0.27±0.10[a]
18:0	-0.47±0.18[a]	-0.57±0.18[a]	-0.29±0.18[a]
18:1	0.93±0.28[a]	0.93±0.28[a]	0.98±0.28[a]
18:2	0.51±0.18[a]	0.37±0.18[a]	0.80±0.18[a]
18:3	0.02±0.03[a]	0.01±0.03[a]	0.06±0.03[a]
20:3	0.02±0.06[a]	-0.01±0.06[a]	-0.09±0.06[a]
20:4	1.43±0.76[a]	-0.19±0.76[a]	0.32±0.76[a]
20:5	-0.07±0.07[a]	0.07±0.07[a]	0.51±0.07[b]
22:4	-0.24±1.15[a]	-1.67±1.15[a]	-1.95±1.15[a]
22:5	-1.53±0.42[a]	-0.47±0.42[a]	0.89±0.42[b]
22:6	-0.33±0.24[a]	-0.41±0.24[a]	-0.19±0.24[a]
n-3 PUFA[4]	-1.92±0.66[a]	-0.82±0.66[a]	1.21±0.66[b]
n-6 PUFA[5]	1.51±1.15[a]	-2.05±1.15[a]	-1.84±1.15[a]

[1] All values are means ± SE and expressed as a % of total fatty acids in the PPE fraction. Platelet PPE data for individual subjects are shown in Appendix 16. A sample fatty acid chromatogram for platelet PPE is shown in Appendix 10.

[2] Carbon chain length:number of double bonds.

[3] Values in rows with the same superscript letter do not differ ($p<0.05$). p-Values for the statistical comparison among the experimental diets are shown in Appendix 24.

[4] n-3 = 20:5 + 22:5 + 22:6.

[5] n-6 = 20:3 + 20:4 + 22:4.

Table 17. Mean Platelet Phosphatidylcholine Fatty Acid Levels on the Mixed Fat and Experimental Diets[1]

Fatty Acid[2]	DIET			
	MF n=24	LLNA n=8	CAN n=8	FLAX n=8
16:0	31.35±0.56[3]	32.24±1.21	31.70±0.57	31.63±0.58
18:0	15.07±0.28	12.54±0.50	13.35±0.43	13.53±0.28
18:1	26.56±0.31	27.51±0.33	27.35±0.41	26.02±0.27
18:2	8.08±0.11	9.71±0.21	9.69±0.25	10.02±0.37
18:3	0.06±0.01	0.12±0.02	0.17±0.01	0.27±0.02
20:2	0.41±0.01	0.59±0.02	0.61±0.02	0.64±0.03
20:3	1.53±0.05	1.21±0.05	1.15±0.04	1.18±0.06
20:4	11.80±0.31	9.82±0.55	9.65±0.39	10.13±0.28
20:5	0.17±0.01	0.13±0.02	0.17±0.02	0.21±0.01
22:4	0.82±0.03	0.75±0.03	0.57±0.09	0.74±0.05
22:5	0.53±0.02	0.39±0.03	0.41±0.02	0.54±0.03
22:6	0.50±0.04	0.39±0.04	0.42±0.05	0.45±0.03
n-3 PUFA[4]	1.19±0.06	0.91±0.09	1.00±0.08	1.19±0.04
n-6 PUFA[5]	14.15±0.33	11.78±0.58	11.37±0.41	12.05±0.26

[1] A table of the mean values on each of the diets. Conclusions were not based on these values but on the changes that occurred when subjects were changed from the MF to the experimental diets. Refer to Tables 11 and 14 for the statistical analysis of the diet effects.
[2] Carbon chain length:number of double bonds.
[3] All values are means ± SE and expressed as a % of total fatty acids in the PC fraction.
[4] n-3 = 20:5 + 22:5 + 22:6.
[5] n-6 = 20:3 + 20:4 + 22:4.

Table 18. Mean Platelet Phosphatidylethanolamine Fatty Acid Levels On the Mixed Fat and Experimental Diets[1]

Fatty Acid[2]	DIET			
	MF n=24	LLNA n=8	CAN n=8	FLAX n=8
16:0	7.75±0.27[3]	7.22±0.36	6.72±0.40	6.92±0.30
18:0	34.31±0.44	30.97±0.71	31.73±0.46	31.65±0.51
18:1	13.88±0.30	20.58±0.58	20.02±0.55	18.83±0.36
18:2	4.01±0.09	5.39±0.26	5.34±0.22	5.82±0.21
18:3	0.03±0.01	0.09±0.02	0.14±0.02	0.17±0.02
20:2	0.25±0.03	0.63±0.21	0.41±0.01	0.42±0.02
20:3	0.97±0.05	1.14±0.08	1.13±0.07	1.13±0.08
20:4	31.20±0.42	26.20±0.48	26.35±1.01	27.00±0.61
20:5	0.20±0.01	0.14±0.02	0.21±0.03	0.28±0.02
22:4	2.11±0.07	1.95±0.07	1.77±0.11	1.63±0.26
22:5	1.41±0.04	1.00±0.07	1.12±0.08	1.44±0.09
22:6	1.28±0.08	1.07±0.09	1.22±0.16	1.21±0.08
n-3 PUFA[4]	2.89±0.11	2.21±0.13	2.55±0.25	2.93±0.14
n-6 PUFA[5]	34.28±0.48	29.28±0.44	29.25±1.05	29.75±0.68

[1] A table of the mean values on each of the diets. Conclusions were not based on these values but on the changes that ocurred when subjects were changed from the MF diet to the experimental diets. Refer to Tables 12 and 15 for the statistical analysis of the diet effects.
[2] Carbon chain length:number of double bonds.
[3] All values are mean ± SE and expressed as a % of total fatty acids in the PE fraction.
[4] n-3 = 20:5 + 22:5 + 22:6.
[5] n-6 = 20:3 + 20:4 + 22:4.

Table 19. Mean Platelet Alkenylacyl Ethanolamine Phosphoglyceride Fatty Acid Levels on the Mixed Fat and Experimental Diets[1]

Fatty Acid[2]	DIET			
	MF n=24	LLNA n=8	CAN n=8	FLAX n=8
16:0	0.32±0.07[3]	0.16±0.08	0.22±0.05	0.13±0.08
18:0	0.94±0.14	0.53±0.11	0.53±0.09	0.43±0.08
18:1	2.30±0.13	3.26±0.25	3.40±0.20	3.08±0.19
18:2	1.29±0.09	1.78±0.20	1.76±0.13	2.00±0.21
18:3	0.04±0.01	0.06±0.02	0.04±0.01	0.09±0.03
20:3	0.78±0.04	0.79±0.07	0.78±0.06	0.70±0.11
20:4	67.85±0.53	69.07±0.71	68.18±0.74	67.84±0.64
20:5	0.79±0.05	0.69±0.05	1.01±0.11	1.18±0.05
22:4	12.69±0.43	12.96±0.49	11.60±0.61	11.27±0.58
22:5	7.78±0.28	6.24±0.40	7.19±0.17	8.80±0.31
22:6	3.92±0.24	3.41±0.29	3.84±0.37	3.57±0.28
n-3 PUFA[4]	12.49±0.48	10.34±0.60	12.04±0.58	13.56±0.42
n-6 PUFA[5]	81.32±0.49	82.82±0.84	80.56±0.61	79.81±0.49

[1] A table of the mean values on each of the diets. Conclusions were not based on these values but on the changes that occurred when the subjects were changed from the MF diet to the experimental diets. Refer to Tables 13 and 16 for the statistical analysis of the diet effects.
[2] Carbon chain length:number of double bonds.
[3] All values are means ± SE and expressed as a % of total fatty acids in the PPE fraction.
[4] n-3 = 20:5 + 22:5 + 22:6.
[5] n-6 = 20:3 + 20:4 + 22:4.

exceptions: STEA levels and total n-6 LC PUFA levels were higher in the PC fraction; PMA and DHA levels were lower while total n-6 LC PUFA levels were higher in the PE fraction; and LA levels were higher and DHA levels were lower in the PPE fraction following the washout phase compared to levels following the pre-experimental phase.

4.4 Summary

There was a significant decrease in plasma TC levels, which were paralleled by a decrease in LDL-C levels, when subjects were changed from the MF to the experimental diets. In addition, there was a significant decrease in VLDL-C levels on the CAN diet while TG and HDL-C levels decreased on the FLAX diet. Changes in plasma and LP cholesterol levels were similar for the experimental diets, relative to the MF diet, even though significant changes were not observed for components on all of the experimental diets (e.g., TG, HDL-C and VLDL-C). Plasma and LP cholesterol levels, but not plasma TG levels, returned to pre-experimental levels when subjects were returned to the MF diet during the washout phase of the study. Plasma TG levels were slightly higher at the end of the washout phase compared to the end of the pre-experimental phase.

Fatty acid patterns of platelet PL changed considerably in response to the experimental diets when compared to levels on the MF diet. The level of PMA decreased in the PPE

Table 20. Mean Differences in Platelet Phosphatidylcholine Fatty Acid Levels Following the Washout and Pre-Experimental Phases (Day 44 vs. Day 8)

Fatty Acid[2]	Mean Difference (% of total fatty acids)	Probability (p>t)
16:0	-0.64±0.65[1]	0.34
18:0	0.63±0.22†	0.01
18:1	-0.47±0.50	0.37
18:2	0.17±0.15	0.30
18:3	0.02±0.01	0.07
20:2	-0.01±0.01	0.54
20:3	0.02±0.05	0.70
20:4	-0.21±0.48	0.67
20:5	-0.03±0.02	0.08
22:4	0.04±0.04	0.30
22:5	-0.01±0.03	0.86
22:6	-0.10±0.05	0.06
n-3 PUFA[3]	0.07±0.07	0.39
n-6 PUFA[4]	2.27±0.46†	0.00

[1] All values are means ± SE.
[2] Carbon chain length:number of double bonds.
[3] n-3 = 20:5 + 22:5 + 22:6.
[4] n-6 = 20:4 + 22:3 + 22:4.
† Values are significantly different ($p<0.05$) from values following pre-experimental period (day 8).

Table 21. Mean Differences in Platelet Phosphatidylethanolamine Fatty Acid Levels Following the Washout and Pre-Experimental Phases (Day 44 vs. Day 8)

Fatty Acid[2]	Mean Difference (% of total fatty acids)	Probability (p>t)
16:0	-1.07±0.33[1†]	0.01
18:0	1.86±0.93	0.07
18:1	-0.75±0.59	0.23
18:2	-0.08±0.09	0.38
18:3	-0.00±0.02	0.97
20:2	0.09±0.06	0.22
20:3	-0.08±0.07	0.28
20:4	0.06±0.81	0.94
20:5	-0.01±0.02	0.80
22:4	0.10±0.09	0.29
22:5	0.06±0.08	0.49
22:6	-0.21±0.08†	0.03
n-3 PUFA[3]	0.09±0.16	0.59
n-6 PUFA[4]	4.29±0.72†	0.00

[1] All values are means ± SE.
[2] Carbon chain length:number of double bonds.
[3] n-3 = 20:5 + 22:5 + 22:6.
[4] n-6 = 20:3 + 20:4 + 22:4.
† Values are significantly different ($p<0.05$) from values following pre-experimental period (day 8).

Table 22. Mean Differences in Platelet Alkenylacyl Ethanolamine Phosphoglyceride Fatty Acid Levels Following the Washout and Pre-Experimental Phases (Day 44 vs. Day 8)

Fatty Acid[2]	Mean Difference (% of total fatty acids)	Probability (p>t)
16:0	0.08±0.17[1]	0.65
18:0	-0.08±0.24	0.73
18:1	0.18±0.23	0.44
18:2	0.30±0.11†	0.02
18:3	-0.03±0.02	0.21
20:3	0.06±0.04	0.14
20:4	1.08±0.62	0.11
20:5	-0.07±0.08	0.41
22:4	-0.28±0.66	0.68
22:5	-0.22±0.34	0.53
22:6	-0.83±0.21†	0.00
n-3 PUFA[3]	-0.31±0.45	0.51
n-6 PUFA[4]	0.91±0.62	0.17

[1] All values are means ± SE.
[2] Carbon chain length:number of double bonds.
[3] n-3 = 20:5 + 22:5 + 22:6.
[4] n-6 = 20:3 + 20:4 + 22:4.
† Values are significantly different ($p<0.05$) from values following pre-experimental period (day 8).

fraction on the FLAX diet and in the PE fraction on the CAN diet only. By contrast, the level of STEA decreased on all of the experimental diets except for in the PPE fraction on the FLAX diet. The level of OA in the PE and PPE fractions increased on all experimental diets, whereas the level in the PC fraction remained unchanged. By contrast, the level of LA increased in all PL fractions on all of the experimental diets except for the PPE fraction where increases of LA were not significant on the CAN diet. The level of LNA increased in PC and PE but was unchanged in PPE as a result of the experimental diets. The level of ETA decreased in PC on the experimental diets; increased in PE on the LLNA and CAN diets; but was unchanged in PPE. Levels of AA and total n-6 LC PUFA followed a similar pattern of change in platelet PL. Thus, both the levels of AA and n-6 LC PUFA decreased in PC and PE but were unchanged in PPE on all experimental diets. The level of DTA decreased in the PC and PE fractions only on the CAN and FLAX diets, respectively. The level of EPA decreased in PE and PPE but was unchanged in PC on the LLNA diet, was unaffected by the CAN diet, but increased in all PL on the FLAX diet. The level of DPA decreased in all PL fractions on the LLNA diet, decreased in PC and PE on the CAN diet, but was unchanged on the FLAX diet. The level of DHA was unaffected by the experimental diets except for the decrease in the PC fraction on the CAN diet. Total n-3 LC PUFA levels decreased in response to the LLNA diet in all PL

fractions but were unaffected by the CAN diet, except for a decrease in the PC fraction. Levels of n-3 LC PUFA increased in response to the FLAX diet, although, the increases were not statistically significant.

In general, changes in the fatty acid composition of platelet PL reflected differences in the fatty acid composition among the experimental diets. Changes in the levels of PMA, STEA and LA were similar among the experimental diets. Likewise, changes in the level of OA also were similar among the experimental diets, although the level of OA was slightly higher on the LLNA and CAN diets than on the FLAX diet in the PE fraction. Changes in the levels of LNA in PC and PE mirrored the amount of LNA found in the experimental diets; increases were greatest on the FLAX diet and lowest on the LLNA diet. Changes in the EPA content of platelet PL, with one exception, were consistently greater on the FLAX diet than on the LLNA or CAN diets. However, decreases in the levels of AA and total n-6 LC PUFA in all PL fractions were similar among the experimental diets, except for in PE where decreases in n-6 LC PUFA levels were greater on the FLAX and LLNA diets. Changes in the levels of DPA were generally smaller in magnitude on the LLNA and CAN diets than on the FLAX diet. Changes in the levels of DHA were similar among the experimental diets.

Following the washout phase of the study, most platelet PL fatty acid levels returned to pre-experimental levels with

the exception of: STEA and total n-6 LC PUFA levels were higher in PC; PMA and DHA which were lower and n-6 LC PUFA which were higher in PE; and LA which was higher and DHA which was lower in PPE. Although these changes in fatty acid levels were statistically significant, they were modest.

5. DISCUSSION

5.1 Plasma Total and Lipoprotein Cholesterol Levels

The experimental diets used in the present study contained equal amounts of LA but differing amounts of LNA, varied at the expense of OA. SFA provided 14% of the total fatty acids in the experimental diets or approximately one-third of the amount in the MF diet. Subjects' plasma and LP cholesterol levels decreased considerably in response to the experimental diets. Plasma TC levels decreased 15% to 21% with a majority of the decrease occurring in the LDL-C fraction which decreased 18% to 23%. By contrast, the experimental diets had minor effects on VLDL-C, HDL-C and TG levels; there was a significant decrease in the levels of VLDL-C (-70%) on the CAN diet and a significant decrease in HDL-C and TG levels (-9% & -17%, resp.) on the FLAX diet. All changes in total and LP cholesterol levels, however, followed the same pattern (statistically similar; ($p<0.05$) regardless of the experimental diet. Previous investigators have reported similar results in response to diets containing high amounts of LA or OA and low amounts of SFA (Chan et al., 1991; McDonald et al., 1989; Mattson and Grundy, 1985; Mensick and Katan, 1985; Snook and Wardlaw, 1988; Shepard et al., 1980; Vega et al., 1982; Ginsberg et al., 1990; Barr et al., 1992; Iacono and Dougherty, 1991; Valsta et al., 1992). In general, these studies found that dietary LA and OA reduced plasma cholesterol mainly through a reduction in LDL-C levels with

little or no effects on HDL-C levels. Reductions have ranged from 12% to 21% in TC and from 12% to 35% in LDL-C levels, which are similar in magnitude to the changes in the present study. By contrast, the responses to dietary LNA have been less consistent. For example, Sanders and Roshanai (1983) and Mest et al. (1983) found that a linseed oil supplement did not lower plasma and LP cholesterol levels in normolipidemic subjects. However, dietary fat intake of the subjects was not monitored in these studies. Nevertheless, evidence from carefully controlled metabolic studies support a hypocholesterolemic effect for linseed oil and for canola oil, which contain appreciable amounts of LNA (Kestin et al., 1990; McDonald et al., 1989; Chan et al., 1991; Chard, 1991; Lasserre et al. 1985; Valsta et al., 1992). Furthermore, the effect of LNA-containing oils on plasma lipid and LP levels has been similar to that of LA and OA-rich oils, i.e., mainly a reduction in TC and LDL-C levels.

The hypercholesterolemic effect of SFA has been shown repeatedly in humans (Keys et al., 1957; Hegsted et al., 1965; Sheperd et al., 1980; Vega et al., 1982; Turner et al., 1981; Ginsberg et al., 1990; Barr et al., 1992) and in animals (Spady and Dietschy, 1988). In addition, substitution of PUFA for SFA in the diet consistently leads to a reduction in plasma cholesterol levels. Thus, the results of the present study are not surprising. Nonetheless, the mechanism or mechanisms responsible for alterations in cholesterol

metabolism in response to changes in dietary fat source remain controversial. In humans, Sheperd et al. (1980) found that decreases in TC and LDL-C levels on LA-containing diets were associated with increases in the fractional catabolic rate (FCR) of the apoprotein B (apoLDL) associated with the LDL molecule. However, other studies found that the FCR of LDL did not change with LA-containing diets (Cortese et al., 1983; Turner et al., 1981). Grundy et al. (1985) proposed that dietary SFA increase the levels of LDL either by decreasing the catabolism of LDL or by increasing the production of LDL. Grundy (1987) subsequently concluded that dietary SFA increase LDL levels by actions similar to dietary cholesterol. He proposed that SFA increase the hepatic content of cholesterol which thereby causes a reduction in the activity of hepatic LDL receptors (Grundy, 1987). The observations by Spady and Dietschy (1988) support the latter mechanism; SFA (coconut oil) augmented the suppressive action of dietary cholesterol on hepatic LDL receptor activity in the hamster while unSFA (olive oil or safflower oil) reduced the suppressive action of dietary cholesterol on LDL receptors.

There is some evidence to support a unique cholesterol-lowering effect of PUFA. Early work conducted by Keys et al. (1957) and Hegsted et al. (1965) found dietary SFA to be approximately twice as active in raising plasma cholesterol as LA was in lowering it, whereas OA had no effect on plasma cholesterol. In addition, Grande et al. (1963) found that the

LC PUFA in fish oils were as effective as LA in lowering plasma cholesterol levels. However, these researchers did not explore the mechanism responsible for their observations. Recent evidence suggests that EPA and DHA have little effect on TC levels, whereas they can reduce plasma TG levels quite dramatically (Harris, 1990; Kestin et al. 1991).

Some reports have suggested that decreases in plasma cholesterol concentration in response to a LA-rich diet are related to an increase in fecal sterol excretion (Nestel, 1987). However, Sheperd et al. (1980) and Grundy et al. (1985) found that LA-rich diets did not affect fecal sterol excretion. Grundy et al. (1985) and Soutar (1978) suggested that PUFA diets increase the fluidity of LDL constituent lipids which increases the rate at which LDL particles are catabolized. Indeed, Berlin et al. (1987) found a moderate increase in LDL fluidity by feeding subjects a LA-rich diet. Grundy et al. (1985) also suggested that there may be some changes in LDL composition which predispose some forms of LDL to be more atherogenic than others. Spritz and Mishkel (1969) suggested that PUFA diets reduce the number of circulating LDL particles. However, Grundy (1987) reported that LDL-C levels and the number of LDL particles fell in parallel only when LA was substituted for SFA in the diet. The effect of dietary LNA on the possible factors affecting cholesterol metabolism has not been tested. Thus whether dietary LNA affects cholesterol metabolism through these mechanisms is not known.

Studies conducted in our laboratory demonstrated that diets containing varying absolute and relative amounts of OA, LA and LNA had similar effects on TC and LDL-C levels (McDonald et al., 1989; Chan et al. 1991; Chard, 1991). In these studies the relative proportions of LA, OA and LNA differed, whereas in the current study all experimental diets contained the same amount of LA. All experimental diets contained similar amounts of SFA (15% of total fatty acids) and unSFA (83% of total fatty acids) but they varied in the amount of LNA, which was varied at the expense of OA. Since the experimental diets resulted in similar reductions in plasma lipid levels, it appears that the unSFA exerted their effects on cholesterol metabolism through a common mechanism. It is possible that the substitution of unSFA for SFA in the diets was the factor responsible for the reductions in plasma lipid levels and that the relative contributions of OA, LA and LNA was irrelevant. The work of Ginsberg et al. (1990) and Barr et al. (1992) support the hypothesis that the amount of SFA in the diet is the key factor contributing to high levels of plasma and LDL cholesterol. Thus, the relative contribution of dietary unSFA (viz., OA, LA or LNA), may not be as important as the total contribution of dietary SFA. Other studies suggest that the magnitude of change in plasma cholesterol levels is dependent upon the P:S ratio of the diet (Spady and Dietschy, 1988), but this suggestion does not take into account that OA has been shown to be hypocholesterolemic.

Thus based on the findings from our laboratory and other laboratories, it appears that the contribution of dietary SFA may be the primary factor influencing plasma cholesterol levels.

In the current study, levels of dietary cholesterol were maintained at a level of approximately 300 mg on the MF and the experimental diets, whereas in past studies in our laboratory (McDonald et al., 1989; Chan et al., 1991; Chard, 1991) the experimental diets contained slightly less dietary cholesterol (100 mg) than the MF diet. However, decreases in plasma and LP cholesterol levels were similar in all studies. Thus, the slightly higher levels of cholesterol in the experimental diets of the current study did not affect the hypocholesterolemic effects of the experimental diets. This observation coincides with the findings of Wardlaw and Snook (1988) who demonstrated that dietary cholesterol appeared to have negligible effects on plasma and LP cholesterol levels in the presence of dietary unSFA. However, in the presence of SFA, dietary cholesterol augmented the hypercholesterolemic effects of SFA (Spady and Dietschy, 1988). Evidence from a large number of studies indicates that for every 100 mg decrease in dietary cholesterol, plasma cholesterol levels decrease by 0.06 mmol/L (McNamara, 1990).

LA-rich diets have been found to decrease the level of HDL-C (Mattson and Grundy, 1985; Goodnight et al., 1982; Sheperd et al., 1980). Since HDL-C levels have been found to

be inversely related to CHD (Castelli et al., 1986), there has been some concern about the effects of LA-rich diets on HDL-C levels. For this reason, it has been advised that the P/S ratio of the diet should not exceed 1 (McNamara, 1990). In the current study, HDL-C levels significantly decreased ($p<0.05$) on the FLAX diet from the levels on the MF diet. Thus, decreases in HDL-C levels may have been due to the high P/S ratio of the FLAX diet (2.5). However, there were no differences among the experimental diets with respect to their effect on HDL-C levels when the effects of the experimental diets were compared. The effects of PUFA diets on HDL-C levels have been variable (Nestel, 1987; Goodnight et al., 1982). Results from our laboratory and other laboratories found that HDL-C levels were unaffected by LNA, OA or LA-enriched diets (McDonald et al., 1989; Chan et al., 1990; Valsta et al., 1992; Kestin et al., 1990; Sanders and Roshanai, 1983; Lassere et al., 1985; Ginsberg et al., 1990). The LA content of the FLAX diet was not particularly high and thus was not likely responsible for this effect. Thus the physiological basis for the observed decrease in HDL-C levels on the FLAX diet is unclear. In addition, there is no evidence to show that a decrease in HDL-C levels that accompanies a decrease in LDL-C levels is harmful (Nestel, 1987).

The CAN diet was associated with lower VLDL-C levels ($p<0.01$) than the FLAX and LLNA diets, whereas the FLAX diet

was associated with lower TG levels ($p<0.05$) than the CAN and LLNA diets. The observation that the CAN diet decreased levels of plasma VLDL-C but not TG levels is surprising since TG are contained primarily in VLDL particles and hence a significant reduction in TG levels is usually the result of a decrease in VLDL levels. Thus, the physiological mechanism responsible for this observation is unknown. In earlier studies, diets containing canola oil were somewhat variable with respect to their effect on VLDL-C and TG levels. McDonald et al. (1989) reported that a canola oil diet did not affect VLDL-C or TG levels, while Chan et al. (1991) and Chard (1991) found decreases in VLDL-C and TG levels in normal and mildly hypercholesterolemic subjects, respectively, on a canola oil diet. On the other hand, Valsta et al. (1992) found that a sunflower diet reduced VLDL-C and TG levels to a greater extent than did a rapeseed (canola) oil diet while Lasserre et al. (1985) and Renaud et al. (1986) found that long-term consumption of a rapeseed oil diet did not affect TG levels. Other investigators also found that LNA-containing diets had neglible effects on plasma TG and VLDL-C (Kestin et al., 1991; Mest et al., 1983; Sanders and Roshanai, 1983). Thus it appears that LNA-containing diets do not consistently reduce VLDL-C and TG levels. However, EPA and DHA in fish oils markedly lower plasma TG and VLDL-C levels (Kestin et al., 1991; Sanders and Roshanai, 1983; Harris et al., 1983; Sanders and Hochland, 1983; Harris, 1989). This effect is

thought to occur through a reduction in the hepatic synthesis of VLDL-C and appears to be limited to diets containing EPA and DHA.

A comparison of plasma lipid levels at the end of the washout phase (day 44) to plasma lipid levels at the end of the pre-experimental phase (day 8) indicated that TC, LDL-C, HDL-C and VLDL-C levels were not statistically different. Thus after 18 days on the MF diet, plasma LP cholesterol levels had returned to the levels on day 8. These results coincide with the observations that plasma and LP cholesterol levels have generally reached steady-state within 9-12 days (Corner, 1989; Chan, 1990). By contrast, plasma TG levels were slightly higher ($p<0.006$) at day 44 compared to day 8. There may have been a transient rise in TG levels during the washout period or the higher level may have been simply a day effect. In any case, the mean increase in TG levels was not large (+0.16 mmol/L).

The mechanisms responsible for the observed changes in cholesterol metabolism were not examined in the current study. Thus, the reduction in VLDL-C levels on the CAN diet as well as the reductions in TG and HDL-C levels on the FLAX diet cannot be fully explained. However, it should be noted that the experimental diets had similar effects on all plasma and LP cholesterol fractions even though the changes in all LP cholesterol fractions were not always significant (e.g. VLDL-C, HDL-C and TG levels). Thus the cholesterol-lowering

effects of these diets may be due to a similar mechanism.

The long-term effect of consuming a diet similar to one of the experimental diets would be expected to reduce the risk of developing CHD. The present study suggests that a reduced risk of developing CHD would be mediated through a reduction in plasma cholesterol levels, particularly LDL-C levels, by consuming a diet high in unSFA and low in SFA. Since the experimental diets were generally equal with respect to their influence on plasma lipids and LP, it appears that reducing the dietary intake of SFA may be the most appropriate recommendation at the present time. However, it must be emphasized that the hypercholesterolemic effect of SFA holds only for lauric, myristic and PMA.

5.2 Platelet Phospholipid Fatty Acid Patterns

The MF diet contained almost three times as much SFA, slightly more OA, half the amount of LA and less LNA than the experimental diets. Changes in the fatty acid composition of platelet PL reflected these differences, suggesting that platelet PL fatty acid composition can be significantly altered by diet. In general, levels of SFA (PMA and STEA) decreased while levels of OA, LA and LNA increased in platelet PL when subjects were switched from the MF diet to the experimental diets. Changes in the levels of OA, LA and LNA in platelet PL also reflected the differences in the OA, LA and LNA composition among the experimental diets. For

instance, increases in levels of OA were greater in PE on the LLNA diet compared to the FLAX and CAN diets, which reflected the slightly higher content of OA in the LLNA diet. Likewise, the uniform level of LA in the experimental diets resulted in a similar increase in platelet LA levels when the subjects were switched from the MF diet to the experimental diets. Similar changes in the fatty acid composition of plasma and platelet PL, CE and erythrocyte PL have been reported in response to dietary fat consumption in humans (Corner et al., 1990; Chan et al., 1993; Chard, 1991; Adam et al., 1988; Sanders and Younger, 1981; Sanders and Hochland, 1983; Sanders and Roshanai, 1983; Mest et al., 1983; Budowski et al., 1984; Renaud et al., 1986; Lasserre et al., 1985; Weaver et al., 1990; Brown et al., 1990) and in animals (Marshall and Johnston, 1982; Huang et al., 1989; Lee et al., 1991; Engler et al., 1988). There were significant increases in the LNA content of platelet PC and PE, but not PPE, as a result of the experimental diets. Levels of LNA were: highest on the FLAX diet, intermediate on the CAN diet and lowest on the LLNA diet for platelet PC; higher following the FLAX diet compared to the LLNA and CAN diets for PE; and unchanged and similar among the diets for PPE. The higher levels of LNA in PL following the experimental diets coincides with earlier studies with canola oil diets (Corner et al., 1990; Chan et al., 1993; Chard, 1991).

Dietary LNA is believed to inhibit the metabolism and

incorporation of LA into plasma and membrane PL. Thus increases in the levels of LNA in PL have been associated with decreases in the levels of LA (Mest et al., 1983; Budowski et al., 1984; Corner et al., 1990; Chan et al., 1993) and in some cases, decreases in levels of OA in PL (Adam et al., 1986). Nonetheless, the level of LA in PL is not always reduced as a result of feeding LNA-containing diets (Adam et al., 1986; Lasserre et al., 1985; Renaud et al., 1986). In the present study, platelet levels of OA, LA and LNA increased in response to the experimental diets indicating that increases in the LNA content of platelet PL were not made at the expense of LA nor OA. However, the increases in the LNA content of platelet PL were small compared to the increases in OA and LA. These results confirm earlier studies which found that LNA was poorly incorporated into PL (Adam et al., 1986; Nettleton, 1991; Sanders and Roshanai, 1983; Sanders and Younger, 1981). Emken et al. (1990) reported that LNA was more rapidly oxidized than LA when human subjects were fed PUFA-containing diets. Similarly, Leyton et al. (1987) found that dietary LNA was more readily oxidized than dietary LA and LC PUFA by the rat; the rate of oxidization to carbon dioxide was similar to OA, PMA and STEA. Thus it is possible that LNA in the present experimental diets was preferentially oxidized for energy purposes.

Changes in the levels of fatty acids in platelet PL in response to the experimental diets were generally small

compared to the changes in the fatty acid composition of the diets. Factors responsible for the rather modest changes in the fatty acid composition of platelet PL may be: the rate and competition of fatty acids for elongating and desaturating enzymes; and/or homeostatic mechanisms which selectively control the amount and quality of fatty acids incorporated into membrane lipids. Research supports the idea that the incorporation of fatty acids into membrane lipids is a tightly regulated system and is specific for the different PL species (Hornstra, 1989; Brenner, 1989; Kinsella, 1990; Mori et al., 1987).

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 influencing the amounts of n-3 and n-6 LC PUFA observed in platelet PL. It is believed that the amounts of n-3 and n-6 LC PUFA present in PL following LA and LNA-containing diets are the result of elongation and desaturation mechanisms present in the liver and other tissues (Brenner, 1989). Results from human and animal studies have found that LNA and its higher homologs are more readily desaturated by Δ6- and Δ5-desaturase enzymes than LA and its higher homologs (Christiansen et al., 1991; Emken et al., 1990). However, many researchers have suggested that the abundance of dietary LA in the Western diet places dietary LNA at a disadvantage for mechanisms of elongation and desaturation (Sanders and

Younger, 1981; Leaf and Weber, 1988; Budowski and Crawford, 1985; Dyerberg, 1986; Nestel, 1987; Marshall and Johnson, 1982; Lasserre et al., 1985; Hirati et al., 1990; Kinsella, 1988; Kinsella et al., 1990; Nordoy and Goodnight, 1990; Nettleton, 1991; Hunter, 1991). For example, Sanders and Roshanai (1983) indicated that possibly the high content of LA in the diet of vegans was responsible for the reduced incorporation of LNA and EPA into the plasma and platelet PL of these individuals.

Previous studies have found that the desaturation and elongation of LNA varied with its ratio to LA, in addition to the level of LNA in the diet (Corner et al., 1990; Chan et al., 1993; Weaver et al., 1990; Adam et al., 1988; Lasserre et al., 1985; Budowski and Crawford, 1985). Corner et al. (1990) and Weaver et al. (1990) found that a canola oil diet, with a LA/LNA ratio of 2.6, significantly increased levels of LNA and EPA in plasma and platelet PL, respectively. Chan et al. (1993) found that a diet containing 6.5% of total fatty acids as LNA and a LA/LNA ratio of 6.9 did not result in higher levels of n-3 LC PUFA in plasma nor platelet PL than a diet containing 0.8% LNA and a LA/LNA ratio of 27. By contrast, diets which contained 6.6% and 13.4% of total fatty acids as LNA, with LA/LNA ratios of 3.0 and 2.7, respectively, resulted in a higher levels of n-3 LC PUFA in plasma and platelet PL. Based on these results, Chan et al. (1993) concluded that the dietary LA/LNA ratio influenced the levels of EPA and n-3 LC

PUFA incorporated into PL more profoundly than did the absolute amount of dietary LNA; and that a fairly low dietary LA/LNA ratio (<3) was required to increase the content of n-3 LC PUFA in PL. An increase in the levels of plasma and platelet EPA and n-3 LC PUFA following a diet containing a low LA/LNA ratio would suggest that dietary LNA can effectively compete for the Δ6-desaturase enzyme when levels of dietary LA are low. Diets containing low LA/LNA ratios have resulted in increases in the amount of EPA, but not DHA, in the plasma, platelet and tissue PL of rats (Christiansen et al., 1991; Huang et al., 1987; Lee et al., 1991; Engler et al., 1991; Garg et al., 1989).

In the present study, the LA/LNA ratios of the experimental diets were 6.3, 4.3 and 2.5 for the LLNA, CAN and FLAX diets, respectively. Levels of platelet EPA, DPA and n-3 LC PUFA were consistently higher on the FLAX diet than on the LLNA and CAN diets (Tables 17-19). As a result, levels of EPA were 1.6 and 1.25 times higher in PC, 2 and 1.3 times higher in PE, and 1.7 and 1.2 times higher in PPE on the FLAX diet compared to on the LLNA and CAN diets, respectively. Compared to levels on the MF diet, the greatest increase in EPA levels on the FLAX diet occurred in the PPE fraction of PL. This coincides with other findings which found EPA to be readily incorporated into the PPE fraction of plasma and platelet PL (Corner et al., 1990; Weaver et al., 1990; Chan et al., 1993). Furthermore, the level of platelet EPA in the PPE fraction on

the FLAX diet (1.2% of total fatty acids) was comparable to levels in previous studies where the LA/LNA ratio was <3 (Corner et al., 1990; Weaver et al., 1990; Chan et al., 1993). Levels of DPA were almost double in all PL fractions on the FLAX diet compared to the LLNA and CAN diets. However, even though total n-3 LC PUFA levels were higher on the FLAX diet, the increases were not statistically significant. On the other hand, levels of EPA and n-3 LC PUFA did not changes on the CAN diet (LA/LNA ratio of 4.3), although DPA levels were lower than on the MF diet. By contrast, although the levels of EPA did not change on the LLNA diet, the levels of DPA and LC n-3 PUFA were lower than on the MF diet suggesting that n-3 LC PUFA levels may decrease on a diet with a LA/LNA ratio of 6.3. However, it should be noted that the LA/LNA ratio on the MF diet was 12.0 which suggests that n-3 LC PUFA levels are influenced by more than simply the LA/LNA ratio alone. It is possible that the LA/LNA ratio and the levels of LA and LNA in the diet are important in determining the amount of n-3 LC PUFA in platelet PL since both the LA and LNA levels were appreciably lower (12% and 1% of total fatty acids, resp.) on the MF diet than on the LLNA diet (25% and 4% of total fatty acids, resp.). Thus, low levels of LNA, in the presence of relatively low levels of LA, may not result in low levels of n-3 LC PUFA in platelet PL.

The results of the present study suggest that the conversion of LNA to EPA and n-3 LC PUFA increased on a diet

with a low LA/LNA ratio, but in the presence of a higher LA/LNA ratio (CAN and LLNA diets), this conversion was either reduced or simply maintained. Similar results were found by previous investigators (Adam et al., 1986; Corner et al., 1990; Weaver et al., 1990; Chan et al., 1993). However, there is evidence to indicate that long-term dietary exposure to low levels of LNA (LA/LNA ratio of 6/1) may increase the content of n-3 LC PUFA in plasma and platelet PL (Renaud et al., 1986). Thus, increasing the duration of the present experiment may have resulted in more marked changes in the amounts of EPA and n-3 LC PUFA in platelet PL.

The in vivo conversion of dietary LNA to EPA is claimed to be inefficient in humans (Leaf and Weber, 1988; Budowski and Crawford, 1988; Sanders and Roshanai, 1983; Sanders and Younger, 1981; Nettleton, 1991). It is also believed that LNA is not biologically equivalent to EPA. Thus, LNA must be converted to EPA before any benefical effects, such as a decrease in the thrombogenic tendency of blood platelets, are exerted in the human body (Nettleton, 1991). Nevertheless, the conversion of LNA to EPA has been demonstrated in metabolic studies with canola and linseed oil (Corner et al., 1990; Chan et al., 1993; Chard, 1991; Weaver et al., 1990; Adam et al., 1986; Sanders and Roshanai, 1983; Lasserre et al., 1985; Renaud et al., 1986). However, evidence suggests that administering preformed EPA and DHA is a much more effective means of increasing EPA and DHA in PL than is

reducing the LA/LNA ratio of the diet (Sanders and Younger, 1981; Sanders and Roshanai, 1983; Kestin et al., 1990). Thus it is argued that fish and fish oils are a more effective source of n-3 LC fatty acids than dietary LNA (Nettleton, 1991; Sanders and Roshanai, 1983; Sanders and Younger, 1981; Budowski and Crawford, 1988; Leaf and Weber, 1988; Harris, 1990). Nonetheless, fish and fish oils are not the preferred source of n-3 PUFA for many individuals. The contribution of n-3 PUFA from LNA-containing oils is therefore important.

The effect of the LA/LNA ratio on n-6 LC PUFA levels in platelet PL appears to be somewhat more complex. In the present study, the LA content of platelet PL increased equally in all PL fractions in response to the experimental diets. However, the AA content decreased in the PC and PE fractions but remained unchanged in the PPE fraction on all experimental diets. A similar pattern occurred with n-6 LC PUFA, which decreased on all experimental diets in the PC and PE fractions but was unchanged in the PPE fraction. These observations indicated that the decrease in AA and total n-6 LC PUFA levels was selective among the PL fractions and that fairly low dietary LA/LNA ratios (i.e., 2.5 - 6.3) were needed to reduce the level of AA and n-6 LC PUFA in platelet PL. Corner et al. (1990) indicated that levels of AA and n-6 LC PUFA decreased in the plasma PC fraction only when the LA/LNA ratio of the diet was low (2.6). However, Chan et al. (1993) found that plasma and platelet AA and n-6 LC PUFA levels decreased on a

diet containing a LA/LNA ratio of 2.7 (13.4% of total fatty acids as LNA), whereas a diet with a LA/LNA ratio of 3.0 (6.6% of total fatty acids as LNA) had no effect on AA and n-6 LC PUFA levels. Thus, it appeared that the total amount of LNA in the diet, in addition to the LA/LNA ratio of the diets, influenced the levels of AA and n-6 LC PUFA in PL. In fact, Chan et al. (1993) concluded that the amount of LNA in the diet may influence on n-6 LC PUFA levels moreso than the LA/LNA ratio of the diet. However, the results of the present study indicated that platelet AA and n-6 LC PUFA levels decreased when the LA/LNA ratios of the diets and the amount of LNA (as a % of total fatty acids) in the diets were: 2.5 and 11%; 4.3 and 6%; and 6.3 and 4%, respectively. Therefore, levels of LNA as low as 4% of total fatty acids (LA levels at 25% of fatty acids) and LA/LNA ratios as high as 6.3 reduced levels of n-6 LC PUFA in platelet PL. However, the physiological basis for these results were not explained by the current study and remain to be identified.

Dietary LNA, in contrast to dietary EPA and DHA, generally does not lead to an increase in the level of DHA in PL (Corner et al., 1990; Chan et al., 1993; Chard, 1991; Renaud et al., 1986; Lassere et al., 1985; Sanders and Younger, 1981; Sanders and Roshanai, 1983; Lee et al., 1989; Marshall and Johnson, 1982; Engler et al., 1988; Huang et al., 1988). The low activity of Δ4-desaturase has been reported as being the factor responsible for low levels of DHA (Budowski

and Crawford, 1985; Leaf and Weber, 1988). However, contrasting evidence was reported by Mest et al. (1983) who found an increase in plasma and platelet DHA after four weeks on a linseed oil supplement. In the present study, DHA levels were not affected by the experimental diets except for a decrease in the platelet PC fraction on the CAN diet. However, the effect of the experimental diets on DHA levels did not differ (statistically similar; $p>0.05$). Brown et al. (1991) found that changes in erythrocyte DHA were correlated with the duration of the feeding period irrespective of the amount of DHA in the diet. However, these results were obtained with MaxEPA supplements and therefore may not be relevant to LNA-containing diets. Nevertheless, DHA levels in the platelet PL of the present study may have increased if the duration of the experimental periods had been longer.

The differences in platelet fatty acid composition in response to the experimental diets were small in magnitude. However, the clinical or physiological significance of these alterations may be important. For instance, a small increase in the amount of plasma EPA (<2% of total fatty acids) was associated with reduced platelet sensitivity to collagen (Budowski et al., 1984). Similarly, a decrease in the amount of plasma AA (7.2% to 6.4% of total fatty acids) and an increase in the amount of plasma EPA (0.6 to 0.7% of total fatty acids) were associated with reduced platelet aggregation and increased bleeding time (Renaud et al., 1986). McDonald

et al. (1989) found that an increase in plasma EPA in response to a canola oil diet led to increased bleeding times in healthy subjects. Chan et al. (1993) found that a diet containing a low LA/LNA ratio (2.7), increased the content of plasma EPA, increased 6-keto-$PGF_{1\alpha}$ production and the 6-keto-$PGF_{1\alpha}/TXB_2$ ratio in normolipidemic subjects. However, neither TXB_2 production nor bleeding times were altered by the diet.

The fatty acid composition of platelet PL generally returned to pre-experimental levels following the washout period of 18 days on the MF diet. Only a few fatty acid levels did not return to pre-experimental levels. For example, STEA and total n-6 LC PUFA levels were higher in PC; PMA and DHA levels were lower and total n-6 LC PUFA levels were higher in PE; and LA levels were higher but DHA levels were lower in PPE at day 44 compared to at day 8. By contrast, Mori et al. (1987) found that EPA, DHA and AA levels in PE and PC required six weeks to return to basal levels after cessation of MaxEPA supplementation. Similarly, Brown et al. (1991) found that 18 weeks on a control diet was needed to return erythrocyte EPA to baseline levels and at 18 weeks erythrocyte DHA levels remained high. In the present study, EPA and total n-3 LC PUFA levels, but not DHA levels in PE and PPE, returned to pre-experimental levels within the 18-day washout period. Several possibilities can be advanced to explain why EPA and n-3 LC PUFA levels on the FLAX diet returned to pre-experimental levels, which contrasts with the

results reported by Mori et al. (1987) and Brown et al. (1991): the source of n-3 fatty acids administered (i.e. canola oil and linseed oil vs. fish oils); a smaller magnitude of change in n-3 LC PUFA levels; and the shorter length of dietary treatment. Since DHA is present in meat, the lower levels of DHA at day 44 compared to day 8 may be an indication that the free-living diets of the subjects contained more meat than the study diets. Thus at the end of the pre-experimental period, the subjects' DHA levels still may have been high but after the 44 days on the study diets the levels may have decreased. Similarly, the addition of beef tallow to the MF diet may have contributed to the higher STEA levels in the PC and PE fractions after 18 days of the MF diet (washout period) compared to levels after 7 days of the MF diet (pre-experimental period).

In general, the patterns of fatty acid change in platelet PL on the experimental diets indicated that: LNA was incorporated into platelet PL but not at the expense of LA; LNA was converted to EPA and n-3 LC PUFA while inhibiting the conversion of LA to AA and LC n-6 PUFA only on the FLAX diet; and that changes in n-3 and n-6 LC PUFA levels were selective among the PC, PE and PPE fractions. The selective incorporation of fatty acids into specific PL classes has been reported elsewhere (Lagarde, 1990; Corner et al., 1990; Chan et al., 1993; Weaver et al., 1990; Mori et al., 1987; Adam et al., 1986; Holub et al., 1988). Corner et al. (1990) found

that the PC fraction of plasma PL, in particular, reflected the compositional changes in dietary fat intake while the plasma PE and PPE fractions had smaller magnitudes of change relative to the compositional changes of the study diets. Weaver et al. (1990) found that the PPE fraction of platelet PL responded considerably to changes in dietary fat source. In fact, the platelet PPE fraction appeared to be a main storage site for EPA, DPA, AA, n-6 and n-3 LC PUFA in various studies (Weaver et al., 1990; Corner et al., 1990; Chan et al., 1993; Lagarde, 1990; Holub et al., 1988). Chan et al. (1993) and Weaver et al. (1990) also reported that although the incorporation of fatty acids in plasma and platelet PL may have differed, they appeared to respond similarly to changes in dietary fatty acid composition. In the present study, platelet PPE appeared to be more stable to changes in dietary fat source, although this fraction readily incorporated EPA. On the other hand, platelet PC and PE responded more readily to changes in fatty acid composition of fatty acids 18 carbons or less.

To summarize the results of the present study, it appeared that: (1) low LNA canola oil is equally hypocholesterolemic to regular canola oil and a mixture of canola, sunflower and flax oils, indicating that dietary unSFA are equally hypocholesterolemic and that these fatty acids may share a common mechanism in reducing plasma lipid levels; (2) a low dietary LA/LNA ratio (FLAX) increased the levels of EPA

and n-3 LC PUFA while decreasing the levels of AA and LC n-6 PUFA in platelet PL, suggesting that the conversion of LA to AA was inhibited while the conversion of LNA to EPA was increased; and (3) slightly higher dietary LA/LNA ratios also reduced levels of AA and n-6 LC PUFA but either decreased (LLNA) or did not affect (CAN) levels of EPA and n-3 LC PUFA in platelet PL, suggesting that the conversion of LA to AA was reduced while the conversion of LNA to EPA was either maintained or only slightly reduced; and (4) LA, rather than EPA and n-3 LC PUFA, may have replaced n-6 and n-3 LC PUFA levels when the dietary ratios were slightly higher.

6. SUMMARY AND CONCLUSIONS

Dietary fatty acids are known to influence blood cholesterol levels and therefore, the risk of developing CHD. Epidemiological and experimental evidence have clearly demonstrated the hypercholesterolemic effect of SFA and the hypocholesterolemic effects of OA and LA. The hypocholesterolemic effect of canola oil, which contains predominantly OA and LNA, has been shown in our and other laboratories. The cholesterolemic effect of low-LNA canola oil, which contains less LNA and more OA and LA than traditional canola oil, has not been documented.

The purpose of the present study was to examine the effect of low LNA canola oil on the plasma and LP levels and the importance of the LA/LNA ratio on the fatty acid composition of platelet PL in healthy young men. A 61-day study was divided into a 7-day pre-experimental period followed by two 18-day experimental periods, which were separated by an 18-day washout period. During the pre-experimental and washout periods, subjects received a MF diet which was similar to that used in previous studies (Corner, 1989; Chan, 1990; Chard, 1991). During the experimental periods, four subjects were randomly assigned to receive two of three experimental diets: i) 100% low linolenic canola oil diet (LLNA); ii) 85% regular canola oil and 15% sunflower oil (CAN); or iii) 67% regular canola oil, 15% flax oil and 18% sunflower oil (FLAX). All diets were identical except for the

sources of added dietary fat. The diets provided approximately 3120 kcalories/day with 48% of calories as CHO, 15% as protein and 33% as fat. The added fat in the diet accounted for approximately 79% of the total dietary fat. The experimental diets contained approximately one-third the level of SFA contained in the MF diet. The amount of LNA and the LA/LNA ratios of the experimental diets were manipulated by varying the proportions of canola, sunflower and flax oils. The percentages of OA and LNA in the experimental diets were: 53% and 4% in the LLNA diet; 51% and 6% in the CAN diet; and 45% and 11% in the FLAX diet. The ratios of dietary LA/LNA in the LLNA, CAN and FLAX diets were: 6.3, 4.3 and 2.5, respectively, whereas the level of LA was constant among the diets (25-27% of total fat).

Blood samples were collected from subjects after a 12-hour fast at the beginning and the end of each diet period. The samples were analyzed for plasma TC, LDL-C, VLDL-C, HDL-C and TG levels and the fatty acid composition of platelet PC, PE and PPE. The results indicated that the experimental diets had similar effects on plasma lipid and LP cholesterol levels. Mean plasma TC and LDL-C levels decreased 18% and 21%, respectively, in response to the experimental diets. The experimental diets had only minor effects on the other plasma LP and TG. HDL-C and TG levels decreased (-9% and -17%, resp.) in response to the FLAX diet only, whereas VLDL-C levels decreased (-70%) on the CAN diet only. All plasma LP

levels returned to pre-experimental levels following the washout phase of the study. Plasma TG levels, however, were slightly higher at day 44 compared to day 8.

Platelet PL fatty acid patterns changed significantly as a result of the experimental diets. In general, levels of OA, LA and LNA increased and levels of STEA decreased from levels on the MF diet. However, changes in the fatty acid composition of the platelet PL varied among the experimental diets. For instance, levels of LNA were increased the greatest on the FLAX diet and the least on the LLNA diet. By contrast, levels of LA increased similarly for the experimental diets which reflected the similar amount of LA in the diets. All experimental diets resulted in decreases in the levels of AA and n-6 LC PUFA in platelet PC and PE, whereas levels of these fatty acids did not change in PPE. However, only the FLAX diet was associated with increases in the levels of EPA and DPA in platelet PL, while these levels decreased on the LLNA diet and were either decreased or unchanged on the CAN diet. Dietary fat source had no effect on the platelet levels of DHA. The level of n-3 LC PUFA tended to increase on the FLAX diet, was unchanged by the CAN diet and decreased in response to the LLNA diet. Most platelet PL levels returned to pre-experimental levels after the washout phase of the study. However, STEA in PC, n-6 LC PUFA levels in PC and PE, and LA levels in PPE were higher, whereas PMA levels in PE and DHA levels in PE and PPE were

lower at day 44 compared to levels on day 8.

The ratio of dietary LA/LNA had a significant effect on the fatty acid composition of platelet PL. A low LA/LNA ratio (2.5) resulted in increased levels of EPA, DPA and total n-3 LC PUFA in platelet PL, while a slightly higher LA/LNA ratio (4.3) had no effect on these levels, and an even higher LA/LNA ratio (6.3) decreased them. AA and total n-6 LC PUFA levels decreased in PC and PE while LA levels increased in all PL in response to all dietary treatments. Thus, when the LA/LNA ratio of the diet was low (2.5), LNA was converted to EPA and LC n-3 PUFA while inhibiting the conversion of LA to AA and LC n-6 PUFA (FLAX). However at slightly higher LA/LNA ratios (4.3 and 6.3), the conversion of LA to AA and LC n-6 PUFA was reduced, while the conversion of LNA to EPA and LC n-3 PUFA was either maintained (CAN) or reduced (LLNA). It therefore appeared that: LA replaced n-3 and n-6 LC PUFA in the platelet PL when the dietary LA/LNA ratio was 6.3; LA replaced n-6 PUFA but not n-3 LC PUFA levels when the LA/LNA ratio was 4.3; and EPA and n-3 LC PUFA replaced AA and n-6 LC PUFA when the LA/LNA ratio was 2.5.

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APPENDICES

Appendix 1. 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 three different dietaryfats on blood lipid patterns and platelet function, 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 several intervals throughout the study. I understand that in addition to venous blood samples, a small cut, less than 1/4 inch in length, will be made, that it will leave a scar and that the tests will require the bleeding to continue for 5 minutes. I also understand that this technique will be carried out four times during the period of the research.

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

Dated the _____ day of _____19__.

Signature: _____

Witness: _____

Appendix 2. Fatty Acid Composition of the Oils Used in the Experimental Diets

Fatty Acid[2]	Oils[1]			
	Low Linolenic Canola	Canola	Sunflower[4]	Flax
16:0	4.4[3]	4.2	6.1	5.8
16:1	0.2	0.2	0.1	0.1
18:0	2.4	2.0	5.7	4.3
18:1	57.1	61.3	19.2	16.7
18:2	29.5	20.4	67.0	17.4
18:3n-6	0.1	0.5	tr	0.2
18:3n-3	3.1	7.7	0.4	54.9
20:0	0.7	0.7	0.3	0.1
20:1	1.3	1.6	0.2	0.1
20:2	0.1	0.1	tr	tr
22:0	0.4	0.4	0.8	0.2
22:1	0.2	0.4	tr	-

1 Means of two samples.
2 Carbon chain length:number of fatty acids.
3 Expressed as % of total fatty acids.
4 Means of four samples.

Appendix 3. Fatty Acid Composition of the Diets

Fatty Acid[2]	DIETS[1]			
	MF	LLNA	CAN	FLAX
14:0	4.58[3]	0.74	0.73	0.74
14:1	0.45	0.08	0.08	0.07
16:0	24.07	8.80	8.46	8.81
16:1	1.83	0.75	0.64	0.65
18:0	12.42	3.72	3.76	4.36
18:1	38.87	53.25	51.35	44.50
18:2	11.64	24.93	25.67	26.85
18:3n6	0.05	0.20	0.36	0.26
18:3n3	0.84	3.95	5.61	10.82
20:0	0.31	0.61	0.58	0.49
20:1	0.51	1.17	1.21	0.95
20:2	0.09	0.10	0.09	0.08
22:0	0.11	0.34	0.36	0.35
22:1	0.08	0.21	0.30	0.21

[1] Means of two duplicate diet samples (one of each menu).
[2] Carbon chain length:number of double bonds.
[3] Expressed as a % of total fatty acids.

Appendix 4. Recipes

Granola

Yield: 58 servings

1 kg	rolled oats
125 g	bran
700 g	oil/fat mix
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
150 g	brown sugar
4 ml	vanilla
4.5 g	baking soda
50 ml	boiling water

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

Chili

Yield: 1 serving

75 g	cooked ground beef
21 g	oil/fat mix
130 g	canned tomatoes, drained
55 g	kidney beans, drained
3 g	dehydrated onions
1.5 g	Worchestershire sauce
2.5 g	chili powder

Place ingredients into individual casseroles. Mix well. Cover and bake at 350°F for 30 minutes.

Appendix 4 (cont'd).

Jellied Fruit

Yield: 1 serving

100 g	jello (prepared)
125 g	canned fruit

Prepare jello according to package directions. Place fruit and jello into individual custard cups. Stir and refrigerate.

Baked Rice

Yield: 1 serving

40 g	rice
13 g	oil/fat mix
75 ml	boiling water
1 ml	salt

Place rice, boiling water and salt into individual casseroles. Cover and bake at 350°F for 30 minutes. Add fat/oil to hot rice.
Fluff with fork.

Kernel Corn

Yield: 1 serving

125 g	Kernel corn (frozen)
6 g	oil/fat mix

Weigh corn into individual casseroles and microwave corn 3 minutes. Remove and add oil/fat mix. Stir.

Scalloped Potatoes

Yield: 1 serving

35 g	potato flakes
90 g	chicken broth (hot)
25 g	oil/fat mix
2 g	onion (dehydrated)
3.5 g	flour
100 ml	boiling water

Place onion, flour, oil/fat and boiling water into individual casseroles. Mix to dissolve flour. Add potato flakes and chicken broth. Mix until potatoes are dispersed. Bake uncovered at 350°F for 40-45 minutes. Stir once during baking and once before serving.

Appendix 4 (cont'd).

Chicken Casserole — Yield: 1 serving

75 g	cooked chicken breast (diced)
15 g	oil/fat mix
35 g	celery (diced)
45 g	canned mushrooms (drained)
50 ml	chicken broth

Place ingredients into individual casseroles. Cover and bake at 350°F for 30 minutes.

Noodles — Yield: 1 serving

30 g	noodles
125 ml	boiling water
4 g	oil

Bring water to boil. Place noodles in pot and bring to boil. Reduce heat and cook for 7-10 minutes. Drain. Transfer to individual casseroles. Add oil. Stir. Add oil. Stir.

---

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

Appendix 5. GC Conditions for Diet and Platelet Lipid Analyses

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

GC Conditions:

- injector and detector temperatures 250°C
- oven temperature 205°C, isothermal
- column head pressure 18 psi
- He flow 50 ml/min (Diet analysis); 30 ml/min (platelet lipid analysis) (15 - 22 ml/min. overnight)
- gas gauge settings: Air 50 psi
  Hydrogen 60 psi
  Nitrogen 60 psi
  Helium 30 or 50 psi
- other GC settings: Range 2
  Zero OFF
  Attenuation 0
  Purge B ON
  Oven Max 230°C

Appendices 6 - 10. (Pages 131-138)

Chromatograms of the Fatty Acid Standards and Sample Fatty Acid Chromatograms of the Diet and Platelet Analyses are available at the Elizabeth Dafoe Library, University of Manitoba.

Appendix 11. Formula for Adjusting Chromatogram Peak Areas for Contamination

Fatty Acid Calculations (with correction for blank)

1. On blank chromatograms, mark 15:0, 16:0 and 18:0 peaks (mark 18:1 if area is significant). Transfer AREA values to data sheet. Average these AREA values for each fatty acid (FA); use average values to correct for contamination (C).

2. On sample chromatogram, mark 15:0 standard and all FA of interest (17 FA).

3. Correct the 16:0 and 18:0 AREA% values on sample chromatogram:

   a) for PE and PPE:

$$C = \frac{\text{AREA FA (blank)}}{\text{AREA 15:0 (blank)}} \times \text{AREA 15:0 (sample)}$$

   for PC:

$$C = \frac{\text{AREA FA (blank)}}{\text{AREA 15:0 (blank)}} \times 0.5 \times \text{AREA 15:0 (sample)}$$

   b) for all fractions:

$$\text{corrected AREA\%} = \frac{\text{AREA FA (sample)} - C}{\text{total chromatogram area}} \times 100$$

4. Total the AREA% values for the FA of interest.

5. For each FA, calculate AREA% values to 100%. Enter the new AREA% values on the data sheet:

$$\text{New AREA\%} = \frac{\text{FA AREA\%}}{\text{total AREA\% for FA of interest}} \times 100$$

Appendix 12
A. SAS Program for the ANOVA Combined Analysis

For Platelet Phospholipids:

PART I - Intra-analysis

```
1.  DATA PHOSPHO;
2.  INPUT SUBJECT:2. PL:$3. DAY DIET:$2. VAR16_0 VAR18_0
    ... VAR22_6;
3.  DVAR16_0 = VAR16_0 - LAG(16_0);
4.  DVAR18_0 = VAR18_0 - LAG(18_0);
.
.
.
5.  DOMG3 = (VAR20:5 + VAR22:5 + VAR22:6) - LAG(VAR20:5 +
    VAR22:5 + VAR22:6);
6.  DOMG6 = (VAR20:3 + VAR20:4 + VAR22:4) - LAG(VAR20:3 +
    VAR20:4 + VAR22:4);
7.  IF (DAY=26) OR (DAY=62) THEN OUTPUT;
8.  CARDS;
     .....
     .....
9.  DATA ONE;
10. SET PHOSPHO;
11. IF PL="PC" (or PE or PPPE);
12. PROC GLM DATA=ONE;
13. TITLE3 "INTRA-BLOCK ANALYSIS -- FOR TREATMENT(ADJ)";
14. TITLE4 "FOR PL=PC (or PE or PPE)";
15. CLASS DIET SUBJECT;
16. MODEL DVAR16_0 DVAR18_0 ... = DIET SUBJECT;
17. RANDOM SUBJECT;
18. LSMEANS DIET/STDERR PDIFF;
19. ESTIMATE "EFFECT OF DIET LL" DIET 0.66667 -0.33333
    -0.33333;
20. ESTIMATE "EFFECT OF DIET 67" DIET -0.33333 0.66667
    -0.33333;
21. ESTIMATE "EFFECT OF DIET 85" DIET -0.33333 -0.33333
    -0.66667;
22. ESTIMATE "LL VS. 67" DIET 1 -1 0;
23. ESTIMATE "LL VS. 85" DIET 1 0 -1;
24. PROC GLM DATA=ONE;
25. TITLE3 "INTRA-BLOCK ANALYSIS -- FOR BLOCK(ADJ);
26. CLASS DIET SUBJECT;
27. MODEL DVAR16_0 DVAR18_0 ... = DIET SUBJECT;
```

PART II - Inter-analysis

```
28. DATA TOTALS;
29. SET ONE;
30. BY SUBJECT;
```

Appendix 12 A. (cont'd).

```
31. IF FIRST.SUBJECT THEN DO;
32. TOTAL1=0; TOTAL2=0; ...
33. MU=??;
34. DIET1=0; DIET2=0; DIET3=0;
35. RETAIN TOTAL1 TOTAL2 ... MU DIET1 DIET2 DIET3;
36. END;
37. MU=MU+1;
38. TOTAL1= TOTAL1 + DVAR16_0;
39. TOTAL2= TOTAL2 + DVAR18_0;
.
.
40. IF DIET="LL" THEN DIET1=1;
41. IF DIET="67" THEN DIET2=1;
42. IF DIET="85" THEN DIET3=1;
43. IF LAST.SUBJECT THEN DO;
44. KEEP TOTAL1 TOTAL2 ... MU DIET1 DIET2 DIET3;
45. PROC PRINT DATA=TOTALS;
46. TITLE3 "INTER-BLOCK ANALYSIS";
47. TITLE4 "BLOCK TOTALS AND INDICATOR VARIABLES";
48. PROC REG DATA=TOTALS OUTEST=REGEST COVOUT;
49. MODEL TOTAL1 TOTAL2 ... = MU DIET1 DIET2 DIET3/NOINT COVB;
50. RESTRICT DIET1 + DIET2 + DIET3 = 0;

PART III - Combined Intra- and Inter-Analysis

51. proc iml;
52. title3 "combined Intra- and Inter-block Analysis";
53. title4;
54. use two var_all_;
55. read all var{ subject diet y };
56. y=y;
57. n = nrow(y);
58. mse = ???;      /* from intra-block analysis (above) */
59. varb = ???;    /* based on MS(blocks (adj)) */
60. sigma = design(subject) * design(subject)` * varb + i(n)
    * mse;
61. x = design(diet);
62. beta = inv(x` * inv(sigma) * x) * x` * inv(sigma) * y;
63. varbeta = inv(x` * inv(sigma) * x);
64. print "Estimates of Marginal Means", beta varbeta;
65. contrast = { 0.666667 -0.333333 -0.333333
              -0.333333 0.666667 -0.333333
              -0.333333 -0.333333 0.666667};
66. print "Contrast Matrix is " contrast;
67. est = contrast*beta;
68. varest = contrast * varbeta * contrast`;
69. print "Estimated contrast and its covariance", est varest;
70. quit;
```

Appendix 12 A. (cont'd).

For Plasma Lipids and Lipoproteins:

PART I - Intra-Analysis

```
1.  //  JOB
2.  // EXEC SAS
3.  //SYSIN DD *
4.  DATA COMBINED;
5.  INPUT SUBJECT GROUP:$4. DAY DIET:$2. TG TC HDL LDL VLDL;
6.  CARDS;
     ......
7.  PROC PRINT DATA=COMBINED;
8.  PROC SORT DATA=COMBINED;
9.  BY DIET;
10. PROC MEANS DATA=COMBINED N MEAN STDERR;
11. BY DIET;
12. VAR TG TC HDL LDL VLDL;
```

PART II - Inter-analysis

```
13. DATA COMBINED;
14. INPUT (as above);
15. DTG = TG - LAG(TG);
16. DTC = TC - LAG(TC);
17. DHDL = HDL - LAG(HDL);
.
.
18. IF (DAY=26) OR (DAY=62) THEN OUTPUT;
19. CARDS;
     .....
20. DATA TOTALS;
21.  (program follows same outline as above for the remainder
      of inter- and combined analysis)
```

Appendix 12
B. Sample Output of the ANOVA Combined Analysis

The SAS System

The SAS System

estimates of marginal means

BETA	VARBETA		
-0.142445	0.0063181	0.0022887	0.0022887
-0.179234	0.0022887	0.0063181	0.0022887
-0.132071	0.0022887	0.0022887	0.0063181

CONTRAST

contrast matrix is

0.666667	-0.333333	-0.333333
-0.333333	0.666667	-0.333333
-0.333333	-0.333333	0.666667

estimated contrast and its covariance

EST	VAREST		
0.0088052	0.0025863	-0.001343	-0.001343
-0.027985	-0.001343	0.0025863	-0.001343
0.0191789	-0.001343	-0.001343	0.0025863

Appendix 12
C. SAS Program for Analysis of the Washout Phase

For Platelet Phospholipids or Plasma Lipids and Lipoproteins:

```
1.  //  JOB
2.  // EXEC SAS
3.  //SYSIN DD *
4.  DATA BASELINE;
5.  INPUT SUBJECT:2. PL:$3. DAY DIET:$2. VAR 16_0 VAR18_0
    VAR18_1 ... (or TG TC LDL HDL VLDL);
6.  DOMG3 = (VAR20_5 + VAR22_5 + VAR22_6) - LAG(VAR20_5 +
    VAR22_5 + VAR22_6);
7.  DOMG6 = (VAR20_3 + VAR20_4 + VAR22_4) - LAG(VAR20_3 +
    VAR20_4 + VAR22_4);
8.  IF DIET="MF";
9.  IF DAY=8 THEN COUNT=1;
10. IF DAY=44 THEN COUNT=2;
11. CARDS;
     .......
12. PROC SORT DATA=BASELINE;
13. BY PL SUBJECT;
14. DATA PAIRED;
15. ARRAY COMBINE1(2) VAR16_0A VAR16_0B (or TGA TGB);
16. ARRAY COMBINE2(2) VAR18_0A VAR18_0B (or TCA TCB);
17. ARRAY COMBINE3(2) VAR18_1A VAR18_1B (or etc.);
.
18. DO COUNT=1 TO 2;
19. SET BASELINE;
20. BY PL SUBJECT;
21. COMBINE1{COUNT}=VAR16_0 or TG;
22. COMBINE2{COUNT}=VAR18_0 or TC;
23. COMBINE3{COUNT}=VAR18_1 or etc.;
.
24. IF LAST .SUBJECT THEN RETURN;
25. END;
26. DATA DIFFS;
27. SET PAIRED;
28. DIFF16_0=VAR16_0B-VAR16_0A or DIFFTG=TGB-TGA;
29. DIFF18_0=VAR18_0B-VAR18_0B or DIFFTC=TCB-TCA;
30. DIFF18_1=VAR18_1B-VAR18_1B;
.
31. DIFFOMG3=DOMG3B-DOMG3A;
32. DIFFOMG6=DOMG6B-DOMG6A;
33. PROC SORT DATA=DIFFS;
34. BY PL;
35. PROC MEANS N MEAN STDERR T PRT;
36. BY PL;
37. VAR DIFF16_0 DIFF18_0 DIFF18_1 ... DOMG3 DOMG6 or DIFFTG
    DIFFTC, etc.;
38. TITLE "PAIRED T-TESTS";
```

Appendix 12.
D. Sample Output of the Paired t-tests

PAIRED T-TESTS

PL=PPE

Variable	N	Mean	Std Error	T	Prob>|T|
DIFF16_0	12	0.0800000	0.1714775	0.4665334	0.6499
DIFF18_0	12	-0.0841667	0.2420821	-0.3476782	0.7346
DIFF18_1	12	0.1816667	0.2284660	0.7951584	0.4433
DIFF18_2	12	0.3008333	0.1092360	2.7539751	0.0188
DIFF18_3	12	-0.0250000	0.0186880	-1.3377548	0.2080
DIFF20_2	12	0	0	.	.
DIFF20_3	12	0.0625000	0.0388104	1.6103916	0.1356
DIFF20_4	12	1.0800000	0.6150006	1.7560958	0.1068
DIFF20_5	12	-0.0691667	0.0823836	-0.8395684	0.4190
DIFF22_4	12	-0.2808333	0.6630582	-0.4235425	0.6801
DIFF22_5	12	-0.2183333	0.3372883	-0.6473197	0.5307
DIFF22_6	12	-0.8341667	0.2123086	-3.9290293	0.0024
DOMG3	12	-0.3125000	0.4543829	-0.6877459	0.5059
DOMG6	12	0.9100000	0.6247060	1.4566852	0.1731

Appendix 12
E. SAS Program for the Paired t-test Contrasts

```
1.  //  JOB
2.  // EXEC SAS
3.  //SYSIN DD *
4.  DATA TPROB;
5.  INPUT TVALUE FRACTION $ RESPONSE $ CONTRAST $ ;
6.  PROB=(1-PROBT(ABS(TVALUE),9))*2;
7.  CARDS;
     .....
     .....
8.  PROC SORT DATA TPROB;
9.  BY FRACTION RESPONSE;
10. PROC PRINT DATA=TPROB;
```

Appendix 12
F. Sample Output of the Paired t-test Contrasts

The SAS System

OBS	TVALUE	FRACTION	RESPONSE	CONTRAST	PROB
169	0.519	PPE	18:2	LL-85	0.61628
170	2.775	PPE	18:2	LL	0.02158
171	4.365	PPE	18:2	67	0.00181
172	2.042	PPE	18:2	85	0.07153
173	-0.944	PPE	18:3	LL-67	0.36982
174	0.304	PPE	18:3	LL-85	0.76804
175	0.603	PPE	18:3	LL	0.56139
176	1.937	PPE	18:3	67	0.08472
177	0.172	PPE	18:3	85	0.86724
178	1.262	PPE	20:3	LL-67	0.23867
179	0.410	PPE	20:3	LL-85	0.69139
180	0.343	PPE	20:3	LL	0.73947
181	-1.441	PPE	20:3	67	0.18346
182	-0.236	PPE	20:3	85	0.81872
183	1.031	PPE	20:4	LL-67	0.32945
184	1.509	PPE	20:4	LL-85	0.16558
185	1.881	PPE	20:4	LL	0.09266
186	0.423	PPE	20:4	67	0.68222
187	-0.253	PPE	20:4	85	0.80595
188	-5.858	PPE	20:5	LL-67	0.00024
189	-1.342	PPE	20:5	LL-85	0.21247
190	-0.968	PPE	20:5	LL	0.35834
191	7.316	PPE	20:5	67	0.00004
192	0.929	PPE	20:5	85	0.37713
193	1.052	PPE	22:4	LL-67	0.32022
194	-0.102	PPE	22:4	LL-85	0.92099
195	-0.205	PPE	22:4	LL	0.84213
196	-1.693	PPE	22:4	67	0.12470
197	-0.061	PPE	22:4	85	0.95269
198	-4.097	PPE	22:5	LL-67	0.00269
199	-1.803	PPE	22:5	LL-85	0.10489
200	-3.565	PPE	22:5	LL	0.00520
201	2.129	PPE	22:5	67	0.05212
202	-1.115	PPE	22:5	85	0.29374
203	-0.416	PPE	22:6	LL-67	0.68715
204	0.243	PPE	22:6	LL-85	0.81345
205	-1.373	PPE	22:6	LL	0.20299
206	-0.786	PPE	22:6	67	0.45206
207	-1.717	PPE	22:6	85	0.12011

Appendix 13. Plasma Total and Lipoprotein Cholesterol Levels of Individual Subjects

Diet Order Subject Day: 1[1]		TC (mmol/L) 8	 26	 44	 62
FLAX-CAN					
1	4.88	4.45	3.86	4.76	3.77
3	5.55	4.92	3.82	5.07	4.09
FLAX-LLNA					
7	4.99	5.40	4.83	4.87	4.51
11	4.36	4.49	3.34	4.36	3.18
LLNA-CAN					
8	3.86	4.02	3.37	3.71	3.38
12	4.88	4.97	4.32	4.30	3.96
LLNA-FLAX					
5	4.61	4.56	3.69	4.97	3.99
6	4.80	4.34	3.88	4.69	3.40
CAN-LLNA					
2	4.55	3.76	3.49	4.05	3.50
4	4.10	3.66	3.04	4.16	3.51
CAN-FLAX					
6	4.80	4.34	3.88	4.69	3.40
10	4.36	4.49	3.34	4.36	3.18

1 Baseline measurement on Day 1 of study.

Appendix 13 (cont'd).

Diet Order / Subject / Day:	LDL-C (mmol/L) 8	26	44	62
FLAX-CAN				
1	3.00	2.55	2.97	2.22
3	3.94	2.93	3.68	3.08
FLAX-LLNA				
7	3.70	3.60	3.44	3.20
11	2.45	1.68	2.60	1.74
LLNA-CAN				
8	2.63	2.04	2.61	2.19
12	3.75	2.96	3.29	2.92
LLNA-FLAX				
5	3.50	2.72	3.43	2.72
6	2.72	2.51	3.00	2.03
CAN-LLNA				
2	2.60	2.31	2.95	2.17
4	2.37	1.97	2.40	1.98
CAN-FLAX				
9	3.09	2.40	2.98	2.28
10	3.08	2.35	3.19	2.12

Appendix 13 (cont'd).

Diet Order Subject Day:	HDL-C (mmol/L) 8	26	44	62
FLAX-CAN				
1	1.45	1.31	1.50	1.39
3	0.88	0.78	1.02	0.92
FLAX-LLNA				
7	0.91	0.91	0.85	0.87
11	1.42	1.20	1.18	1.11
LLNA-CAN				
8	1.39	1.33	1.10	1.19
12	1.17	1.35	0.95	0.99
LLNA-FLAX				
5	0.76	0.69	0.97	0.76
6	1.40	1.37	1.45	1.01
CAN-LLNA				
2	1.15	1.18	1.02	1.16
4	0.96	0.92	1.18	1.18
CAN-FLAX				
9	1.25	1.22	1.15	1.17
10	1.03	0.99	0.95	1.06

Appendix 13 (cont'd).

Diet Order Subject Day:	VLDL-C (mmol/L) 8	26	44	62
FLAX-CAN				
1	0.00	0.00	0.29	0.16
3	0.10	0.11	0.37	0.09
FLAX-LLNA				
7	0.79	0.32	0.58	0.44
11	0.22	0.03	0.00	0.12
LLNA-CAN				
8	0.00	0.00	0.00	0.00
12	0.05	0.01	0.06	0.05
LLNA-FLAX				
5	0.30	0.28	0.57	0.51
6	0.22	0.00	0.24	0.36
CAN-LLNA				
2	0.01	0.00	0.08	0.17
4	0.33	0.15	0.58	0.35
CAN-FLAX				
9	0.39	0.00	0.21	0.02
10	0.38	0.00	0.22	0.00

Appendix 13 (cont'd).

Diet Order Subject Day:	TG (mmol/L) 8	26	44	62
FLAX-CAN				
1	0.71	0.52	0.80	0.42
3	0.83	0.81	1.06	0.76
FLAX-LLNA				
7	1.33	1.37	1.71	1.65
11	0.63	0.54	0.49	0.59
LLNA-CAN				
8	0.38	0.43	0.51	0.39
12	0.91	0.90	0.80	0.77
LLNA-FLAX				
5	1.49	1.00	1.73	1.10
6	0.61	0.62	0.80	0.96
CAN-LLNA				
2	0.39	0.61	0.53	0.46
4	1.17	0.75	1.51	1.04
CAN-FLAX				
9	0.71	0.64	0.81	0.51
10	0.84	0.77	1.15	0.66

Appendix 14. The Fatty Acid Composition of Platelet Phosphatidylcholine of Individual Subjects

Diet Order / Subject	Fatty Acid[1] (% of total) 16:0				16:1			
Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	31.20	30.74	30.59	31.55	0.37	0.31	0.25	0.25
3	32.01	33.48	27.49	31.99	1.17	1.16	1.00	1.16
FLAX-LLNA								
7	28.29	31.49	31.32	33.75	1.03	0.96	1.37	1.22
11	36.43	31.88	35.21	36.60	0.23	0.70	0.86	0.79
LLNA-CAN								
8	32.59	33.88	34.53	31.75	0.92	0.92	1.08	0.78
12	33.49	33.63	32.88	32.44	0.13	0.87	1.04	0.81
LLNA-FLAX								
5	31.34	34.74	31.06	33.67	0.50	0.12	0.43	0.36
6	30.84	29.74	29.45	30.16	0.91	0.21	0.72	0.53
CAN-LLNA								
2	29.55	30.45	28.55	26.26	0.26	0.24	0.94	0.78
4	20.28	31.50	29.78	29.49	0.30	0.36	1.09	0.99
CAN-FLAX								
9	27.72	29.13	28.39	28.96	0.97	0.22	0.94	0.80
10	37.75	34.77	33.13	32.67	1.33	0.97	1.23	0.82

[1] Carbon chain length:number of double bonds.

Appendix 14 (cont'd).

Diet Order	Fatty Acid (% of total)							
	18:0				18:1			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	15.40	14.38	16.95	14.78	25.19	25.50	24.16	26.35
3	14.90	13.43	16.24	13.11	25.25	25.72	25.01	25.64
FLAX-LLNA								
7	16.82	13.46	16.02	13.09	26.43	25.77	27.43	28.03
11	13.74	13.30	13.79	11.72	25.96	26.40	24.69	25.92
LLNA-CAN								
8	13.23	11.26	12.73	12.01	26.08	27.60	26.31	27.95
12	12.67	11.32	13.68	11.67	28.28	26.95	28.87	27.76
LLNA-FLAX								
5	13.66	11.03	14.60	11.81	26.62	28.28	27.50	26.81
6	14.37	13.18	15.52	14.08	26.07	26.91	24.71	25.41
CAN-LLNA								
2	16.43	15.18	17.71	14.89	28.43	27.86	28.58	28.94
4	15.70	13.60	15.83	13.82	26.38	27.64	26.81	27.47
CAN-FLAX								
9	15.68	13.68	16.49	14.18	26.43	29.28	26.79	27.36
10	14.39	12.77	15.02	13.61	30.35	26.34	25.02	25.20

Appendix 14 (cont'd).

Diet Order	Fatty Acid (% of total)							
	18:2				18:3			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	7.95	10.05	7.98	10.08	0.03	0.26	0.04	0.17
3	6.76	8.29	7.29	8.61	0.06	0.23	0.06	0.18
FLAX-LLNA								
7	8.66	8.55	8.00	9.81	0.05	0.22	0.05	0.12
11	7.99	10.23	8.11	8.95	0.10	0.26	0.19	0.20
LLNA-CAN								
8	8.58	9.82	8.78	10.44	0.04	0.08	0.04	0.16
12	8.47	10.44	8.11	10.48	0.05	0.09	0.05	0.19
LLNA-FLAX								
5	7.95	9.65	8.79	11.16	0.05	0.07	0.04	0.34
6	8.17	9.80	8.56	10.66	0.04	0.08	0.05	0.31
CAN-LLNA								
2	8.07	9.60	7.47	10.43	0.03	0.14	0.04	0.16
4	7.42	8.80	8.21	8.79	0.01	0.15	0.09	0.15
CAN-FLAX								
9	8.21	9.93	9.05	10.70	0.05	0.18	0.06	0.24
10	7.73	9.58	7.60	10.55	0.04	0.18	0.06	0.31

Appendix 14 (cont'd).

Diet Order	Fatty Acid (% of total)							
	20:0				20:1			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	1.03	1.47	1.13	1.47	1.40	2.47	1.32	2.73
3	0.86	1.08	0.98	1.16	1.53	2.18	1.40	2.35
FLAX-LLNA								
7	0.97	1.48	0.99	1.34	1.63	3.11	1.48	2.68
11	0.67	1.11	0.66	0.98	1.09	2.00	1.04	1.86
LLNA-CAN								
8	1.10	1.42	0.85	1.42	1.38	2.61	1.30	2.56
12	0.77	1.33	0.89	1.26	0.98	2.23	1.16	2.19
LLNA-FLAX								
5	0.94	1.18	1.06	1.21	1.14	2.12	1.33	2.07
6	0.96	1.44	1.01	1.49	1.70	2.69	1.46	2.48
CAN-LLNA								
2	0.70	1.30	0.61	1.28	1.30	2.31	1.05	2.53
4	0.94	1.31	0.95	1.41	1.50	2.47	1.50	2.48
CAN-FLAX								
9	1.37	1.46	1.17	1.46	2.09	2.90	1.55	2.56
10	1.02	1.52	1.09	1.61	1.36	2.66	1.34	2.39

Appendix 14 (cont'd).

Diet Order	Fatty Acid (% of total)							
	20:2				20:3			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	0.43	0.74	0.44	0.72	1.66	1.21	1.73	1.14
3	0.41	0.52	0.42	0.57	1.19	0.78	1.52	1.02
FLAX-CAN								
7	0.52	0.70	0.44	0.65	1.89	1.31	1.59	1.20
11	0.34	0.56	0.38	0.48	1.40	1.17	1.47	1.14
LLNA-CAN								
8	0.36	0.57	0.34	0.57	1.21	0.96	1.12	1.00
12	0.35	0.58	0.38	0.55	1.63	1.34	1.55	1.29
LLNA-FLAX								
5	0.32	0.53	0.37	0.54	1.98	1.47	1.81	1.29
6	0.49	0.65	0.42	0.66	1.20	1.10	1.44	1.08
CAN-LLNA								
2	0.38	0.57	0.38	0.64	1.38	1.04	1.33	1.22
4	0.46	0.61	0.43	0.60	1.57	1.26	1.60	1.27
CAN-FLAX								
9	0.48	0.66	0.44	0.67	1.74	1.30	1.67	1.35
10	0.42	0.61	0.43	0.70	1.41	1.17	1.69	1.26

Appendix 14 (cont'd).

Diet Order	Fatty Acid (% of total)							
	20:4				20:5			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	12.83	10.70	12.98	9.06	0.12	0.15	0.11	0.11
3	13.14	10.91	15.51	11.77	0.20	0.24	0.26	0.23
FLAX-LLNA								
7	11.85	10.28	9.59	7.09	0.14	0.23	0.12	0.08
11	10.31	10.29	11.35	9.52	0.18	0.22	0.15	0.14
LLNA-CAN								
8	12.44	9.30	11.26	9.76	0.13	0.09	0.11	0.13
12	11.09	9.44	9.58	9.55	0.16	0.11	0.12	0.15
LLNA-FLAX								
5	12.73	8.98	10.75	9.00	0.18	0.09	0.13	0.18
6	12.85	12.00	14.13	10.98	0.15	0.11	0.16	0.23
CAN-LLNA								
2	11.04	9.42	11.17	10.75	0.32	0.19	0.15	0.18
4	13.13	10.30	11.60	11.46	0.31	0.26	0.21	0.23
CAN-FLAX								
9	12.49	9.41	11.15	9.85	0.24	0.14	0.17	0.17
10	8.91	7.89	11.25	9.00	0.11	0.12	0.14	0.23

Appendix 14 (cont'd).

Diet Order	Fatty Acid (% of total)							
	22:3				22:4			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	0.18	0.13	0.16	0.11	0.78	0.69	0.80	0.58
3	0.26	0.14	0.22	0.15	0.96	0.74	1.02	0.85
FLAX-LLNA								
7	0.13	0.15	0.11	0.07	0.82	1.05	0.79	0.57
11	0.11	0.09	0.24	0.09	0.65	0.69	0.82	0.72
LLNA-CAN								
8	0.09	0.08	0.08	0.73	0.92	0.75	0.73	0.02
12	0.10	0.08	0.08	0.08	0.74	0.68	0.75	0.70
LLNA-FLAX								
5	0.18	0.14	0.14	0.08	1.04	0.81	0.94	0.68
6	0.14	0.13	0.16	0.08	0.94	0.90	1.00	0.77
CAN-LLNA								
2	0.12	0.09	0.11	0.12	0.61	0.54	0.72	0.75
4	0.11	0.07	0.13	0.08	0.83	0.65	0.74	0.81
CAN-FLAX								
9	0.26	0.09	0.14	0.07	0.65	0.58	0.89	0.57
10	0.08	0.05	0.10	0.06	0.68	0.67	0.93	0.71

Appendix 14 (cont'd).

Diet Order	Fatty Acid (% of total)							
	22:5				22:6			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	0.62	0.56	0.65	0.38	0.68	0.57	0.59	0.42
3	0.50	0.47	0.65	0.48	0.70	0.56	0.81	0.63
FLAX-LLNA								
7	0.41	0.66	0.37	0.24	0.26	0.47	0.23	0.17
11	0.45	0.62	0.60	0.47	0.29	0.39	0.36	0.35
LLNA-CAN								
8	0.44	0.29	0.37	0.37	0.39	0.27	0.27	0.25
12	0.50	0.36	0.43	0.41	0.50	0.43	0.36	0.40
LLNA-FLAX								
5	0.63	0.34	0.51	0.46	0.66	0.44	0.43	0.39
6	0.58	0.44	0.60	0.53	0.49	0.51	0.51	0.47
CAN-LLNA								
2	0.52	0.38	0.53	0.44	0.76	0.59	0.56	0.52
4	0.67	0.50	0.56	0.53	0.76	0.43	0.39	0.45
CAN-FLAX								
9	0.69	0.41	0.52	0.49	0.81	0.41	0.48	0.45
10	0.36	0.36	0.51	0.52	0.23	0.23	0.36	0.27

Appendix 15. The Fatty Acid Composition of Platelet Phosphatidylethanolamine of Individual Subjects

Diet Order	Fatty Acid[1] (% of total)							
	16:0				16:1			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	8.06	8.09	7.61	8.22	0.19	0.03	0.19	0.37
3	8.82	6.73	6.50	6.67	0.28	0.17	0.17	0.17
FLAX-LLNA								
7	7.04	6.15	6.13	6.03	0.00	0.11	0.20	0.17
11	8.72	6.75	8.93	8.02	0.21	0.28	0.21	0.34
LLNA-CAN								
8	7.78	7.43	7.96	6.99	0.32	0.37	0.28	0.32
12	11.32	8.98	9.51	8.32	0.27	0.34	0.42	0.22
LLNA-FLAX								
5	8.43	7.65	8.73	8.17	0.25	0.22	0.08	0.23
6	8.57	7.13	6.48	7.20	0.28	0.32	0.29	0.17
CAN-LLNA								
2	7.29	5.43	7.00	6.51	0.29	0.25	0.33	0.26
4	7.23	5.61	5.27	6.01	0.25	0.23	0.62	0.12
CAN-FLAX								
9	9.24	6.89	6.09	6.18	0.56	0.25	0.09	0.20
10	6.97	5.62	6.39	6.09	0.29	0.23	0.31	0.11

[1] Carbon chain length:number of double bonds.

Appendix 15 (cont'd).

Diet Order / Subject	Fatty Acid (% of total) 18:0				18:1			
Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	33.54	32.19	35.77	34.05	13.76	18.73	11.87	20.58
3	34.20	30.83	33.18	30.24	14.24	17.97	12.44	18.13
FLAX-LLNA								
7	32.35	30.29	39.92	34.19	14.55	18.37	11.37	19.20
11	32.97	30.74	35.35	30.99	15.61	18.84	13.54	20.91
LLNA-CAN								
8	32.43	29.46	37.21	31.68	13.17	22.44	15.54	20.49
12	32.94	28.48	35.20	30.81	13.17	21.89	16.98	21.01
LLNA-FLAX								
5	34.65	30.39	32.37	30.12	11.98	20.09	11.51	18.00
6	31.79	29.59	32.90	32.01	15.92	21.31	14.31	21.07
CAN-LLNA								
2	33.15	30.65	35.14	31.07	15.58	20.35	14.34	21.42
4	35.59	31.66	31.64	33.59	13.78	17.21	14.49	17.36
CAN-FLAX								
9	33.83	33.24	39.07	34.35	14.60	20.52	12.65	18.23
10	33.12	31.52	35.07	32.70	14.72	21.90	13.05	19.42

Appendix 15 (cont'd).

Diet Order	Fatty Acid (% of total)							
	18:2				18:3			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	3.57	5.62	3.74	5.28	0.00	0.10	0.00	0.12
3	3.72	4.98	3.53	4.75	0.05	0.24	0.22	0.19
FLAX-LLNA								
7	3.62	5.12	3.02	4.82	0.00	0.22	0.00	0.09
11	4.24	5.89	4.69	5.68	0.00	0.13	0.00	0.09
LLNA-CAN								
8	4.47	6.00	4.26	5.65	0.00	0.07	0.00	0.10
12	4.92	6.66	4.81	6.49	0.06	0.07	0.05	0.14
LLNA-FLAX								
5	3.66	5.48	4.15	6.50	0.03	0.05	0.00	0.18
6	4.27	5.20	4.00	6.28	0.00	0.01	0.00	0.19
CAN-LLNA								
2	3.73	5.13	3.68	5.03	0.00	0.27	0.06	0.25
4	3.97	4.40	3.67	4.25	0.05	0.09	0.00	0.10
CAN-FLAX								
9	4.00	5.43	3.88	5.61	0.06	0.10	0.04	0.14
10	4.43	5.61	4.17	6.57	0.04	0.12	0.03	0.21

Appendix 15 (cont'd).

Diet Order	Fatty Acid (% of total)							
	20:0				20:1			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	0.97	1.29	1.03	1.45	0.94	1.49	0.84	1.83
3	0.72	1.04	1.28	1.08	0.69	1.43	1.42	1.64
FLAX-LLNA								
7	1.02	1.37	1.56	1.68	1.33	1.68	1.02	2.15
11	0.64	1.04	0.76	1.08	0.98	1.54	0.64	1.49
LLNA-CAN								
8	0.97	1.31	1.14	1.50	0.89	1.82	0.94	1.99
12	0.89	1.36	1.03	1.41	0.88	1.69	0.94	1.73
LLNA-FLAX								
5	0.99	1.48	1.04	1.66	0.67	1.73	0.88	1.68
6	0.76	1.08	1.07	1.02	1.01	1.66	1.19	1.38
CAN-LLNA								
2	0.62	1.31	0.58	1.14	0.86	1.63	0.13	1.58
4	0.89	1.32	0.83	1.09	0.81	1.80	1.26	1.39
CAN-FLAX								
9	1.21	1.30	1.20	1.52	0.97	1.76	1.00	1.84
10	1.16	1.64	1.17	1.67	0.86	2.03	0.78	1.70

Appendix 15 (cont'd).

Diet Order	Fatty Acid (% of total)							
	20:2				20:3			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	0.20	0.42	0.23	0.43	1.25	1.24	1.21	1.20
3	0.19	0.37	0.31	0.37	0.75	0.76	0.75	0.87
FLAX-LLNA								
7	0.22	0.47	0.25	0.51	1.27	1.32	1.11	1.33
11	0.18	0.38	0.26	0.39	0.79	0.88	0.99	1.10
LLNA-CAN								
8	0.21	0.43	0.23	0.41	0.86	1.01	0.77	0.91
12	0.21	0.42	0.94	0.41	0.97	1.36	0.25	1.16
LLNA-FLAX								
5	0.16	0.40	0.20	0.44	1.13	1.46	1.25	1.31
6	0.22	0.38	0.24	0.35	0.76	0.92	0.79	0.98
CAN-LLNA								
2	0.20	0.41	0.19	0.40	1.02	1.13	0.80	1.04
4	0.22	0.43	0.21	2.08	0.90	1.06	0.66	0.87
CAN-FLAX								
9	0.29	0.37	0.21	0.44	1.03	1.17	0.91	1.13
10	0.27	0.48	0.25	0.51	1.43	1.55	1.37	1.40

Appendix 15 (cont'd).

Diet Order	Fatty Acid (% of total)							
	20:4				20:5			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	31.73	25.93	32.06	22.80	0.14	0.23	0.15	0.09
3	31.12	30.05	34.43	30.22	0.24	0.33	0.27	0.28
FLAX-LLNA								
7	32.63	28.65	30.55	25.35	0.25	0.34	0.19	0.14
11	30.31	27.80	30.10	25.33	0.21	0.29	0.19	0.17
LLNA-CAN								
8	33.68	25.78	27.74	25.92	0.18	0.12	0.13	0.16
12	29.62	24.58	25.36	24.36	0.20	0.14	0.15	0.17
LLNA-FLAX								
5	32.01	26.08	33.49	26.05	0.20	0.12	0.22	0.26
6	31.38	27.50	33.39	26.66	0.00	0.07	0.20	0.31
CAN-LLNA								
2	30.85	27.55	31.54	26.12	0.32	0.26	0.19	0.16
4	31.24	30.59	34.01	28.87	0.32	0.35	0.33	0.23
CAN-FLAX								
9	28.95	24.97	30.37	26.08	0.24	0.19	0.22	0.22
10	30.48	24.39	31.66	24.76	0.19	0.20	0.18	0.29

Appendix 15 (cont'd).

Diet Order / Subject	Fatty Acid (% of total) 22:3				22:4			
Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	0.44	0.30	0.29	0.26	1.93	1.52	1.82	1.32
3	0.40	0.28	0.38	0.32	1.82	1.84	2.10	2.01
FLAX-LLNA								
7	0.35	0.27	0.24	0.26	2.55	2.39	2.14	2.20
11	0.19	0.24	0.23	0.21	2.06	1.86	1.96	1.90
LLNA-CAN								
8	0.22	0.15	0.12	0.17	2.19	1.82	1.98	1.90
12	0.22	0.15	0.16	0.14	1.79	1.66	1.93	1.57
LLNA-FLAX								
5	0.34	0.28	0.31	0.24	2.35	2.16	2.60	2.14
6	0.26	0.28	0.12	0.31	2.17	2.10	2.35	0.00
CAN-LLNA								
2	0.29	0.31	0.30	0.20	1.80	1.82	2.21	2.01
4	0.16	0.16	0.25	0.15	1.77	2.06	2.55	1.76
CAN-FLAX								
9	0.28	0.16	0.17	0.16	1.65	1.36	1.63	1.44
10	0.38	0.20	0.28	0.16	2.62	2.09	2.65	1.83

Appendix 15 (cont'd).

Diet Order	Fatty Acid (% of total)							
	22:5				22:6			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	1.93	1.30	1.44	0.87	1.66	1.34	1.51	0.90
3	1.08	1.27	1.39	1.27	1.51	1.52	1.63	1.78
FLAX-LLNA								
7	1.46	1.77	1.30	0.92	1.18	1.28	0.76	0.73
11	1.52	1.88	1.64	1.36	1.21	1.27	0.92	1.02
LLNA-CAN								
8	1.45	0.74	0.95	0.92	0.96	0.80	0.54	0.68
12	1.15	0.80	1.12	0.86	1.20	1.21	0.88	0.98
LLNA-FLAX								
5	1.42	0.93	0.85	1.44	1.52	1.28	1.44	1.38
6	1.27	0.98	1.33	1.12	1.15	1.26	1.13	1.06
CAN-LLNA								
2	1.52	1.32	1.66	1.15	2.31	2.01	1.67	1.45
4	1.36	1.46	1.98	1.10	1.25	1.36	1.68	0.82
CAN-FLAX								
9	1.37	0.97	1.31	1.24	1.39	1.09	0.90	0.95
10	1.64	1.26	1.64	1.51	1.23	0.95	1.01	0.85

Appendix 16. The Fatty Acid Composition of Platelet Alkenylacyl Ethanolamine Phosphoglyceride of Individual Subjects

Diet Order / Subject / Day:	Fatty Acid[1] (% of total) 16:0				16:1			
	8	26	44	62	8	26	44	62
FLAX-CAN								
1	0.84	0.15	0.22	0.23	0.69	0.17	0.22	0.38
3	0.15	0.08	0.08	0.34	0.00	0.00	0.00	0.19
FLAX-LLNA								
7	0.00	0.00	0.29	0.00	0.00	0.00	0.00	0.00
11	0.47	0.00	0.00	0.10	0.08	0.00	0.17	0.37
LLNA-CAN								
8	0.00	0.00	1.14	0.35	0.00	0.00	0.00	0.00
12	0.81	0.68	0.05	0.04	0.00	0.23	0.37	0.14
LLNA-FLAX								
5	0.06	0.00	1.17	0.69	0.10	0.10	0.29	0.17
6	0.11	0.26	0.33	0.11	0.32	0.39	0.00	0.00
CAN-LLNA								
2	0.19	0.13	0.10	0.00	0.19	0.21	0.11	0.00
4	0.35	0.34	0.65	0.21	0.15	0.00	1.88	0.00
CAN-FLAX								
9	0.15	0.00	0.15	0.00	0.13	0.10	0.00	0.11
10	0.17	0.36	0.08	0.03	0.00	0.00	0.00	0.13

[1] Carbon chain length:number of double bonds.

Appendix 16 (cont'd).

Diet Order / Subject	Fatty Acid (% of total)							
	18:0				18:1			
Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	0.67	0.25	0.57	0.48	2.54	3.10	2.31	3.44
3	0.67	0.43	0.42	0.57	2.22	2.51	1.62	2.70
FLAX-LLNA								
7	0.00	0.21	1.03	0.85	0.81	2.12	1.83	2.90
11	0.62	0.36	0.41	0.28	2.62	3.51	2.11	4.46
LLNA-CAN								
8	1.71	0.35	2.93	0.95	2.25	2.64	3.29	2.88
12	1.88	1.16	0.36	0.39	2.53	4.20	2.87	3.94
LLNA-FLAX								
5	0.46	0.26	1.07	0.78	1.58	2.71	2.40	3.30
6	0.86	0.54	1.34	0.77	2.34	3.41	3.10	3.66
CAN-LLNA								
2	0.40	0.25	0.53	0.32	2.54	3.72	2.11	2.68
4	1.64	0.84	1.29	0.49	2.53	4.30	3.92	3.08
CAN-FLAX								
9	1.65	0.51	0.60	0.26	2.24	2.92	1.52	2.97
10	1.25	0.26	0.25	0.38	2.30	3.27	1.60	3.44

Appendix 16 (cont'd).

Diet Order	Fatty Acid (% of total)							
	18:2				18:3			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	1.28	2.11	1.62	1.85	0.13	0.06	0.00	0.00
3	0.92	1.25	0.91	1.32	0.05	0.07	0.00	0.00
FLAX-LLNA								
7	0.50	1.08	0.89	1.26	0.00	0.04	0.03	0.12
11	1.36	2.27	1.38	2.82	0.00	0.16	0.07	0.13
LLNA-CAN								
8	0.88	1.34	1.57	1.63	0.01	0.00	0.00	0.06
12	1.40	2.34	2.14	2.36	0.11	0.00	0.04	0.08
LLNA-FLAX								
5	0.93	1.62	1.73	2.37	0.03	0.11	0.05	0.08
6	1.52	1.97	1.93	2.25	0.00	0.03	0.00	0.00
CAN-LLNA								
2	1.27	2.02	1.08	1.33	0.05	0.05	0.00	0.00
4	1.43	2.04	2.11	1.56	0.00	0.05	0.06	0.06
CAN-FLAX								
9	1.08	1.45	1.07	1.79	0.05	0.04	0.00	0.07
10	1.11	1.44	0.86	2.90	0.16	0.06	0.04	0.24

Appendix 16 (cont'd).

Diet Order	Fatty Acid (% of total)							
	20:0				20:1			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	0.11	0.00	0.05	0.00	0.10	0.20	0.10	0.23
3	0.03	0.04	0.00	0.00	0.10	0.17	0.03	0.05
FLAX-CAN								
7	0.06	0.03	0.06	0.11	0.08	0.25	0.13	0.42
11	0.06	0.04	0.04	0.06	0.21	0.18	0.09	0.20
LLNA-CAN								
8	0.09	0.09	0.13	0.08	0.18	0.35	0.21	0.34
12	0.00	0.10	0.03	0.05	0.15	0.29	0.10	0.30
LLNA-FLAX								
5	0.04	0.04	0.10	0.07	0.07	0.22	0.10	0.03
6	0.14	0.06	0.16	0.31	0.22	0.28	0.15	0.26
CAN-LLNA								
2	0.05	0.01	0.00	0.00	0.08	0.19	0.08	0.19
4	0.08	0.07	0.08	0.07	0.10	0.28	0.10	0.19
CAN-FLAX								
9	0.10	0.06	0.05	0.05	0.15	0.24	0.10	0.45
10	0.07	0.04	0.03	0.15	0.15	0.30	0.09	0.40

Appendix 16 (cont'd).

Diet Order	Fatty Acid (% of total)							
	20:3				20:4			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	0.82	0.86	0.88	0.80	65.80	67.77	67.90	69.24
3	0.48	0.47	0.48	0.51	62.73	66.51	66.81	66.77
FLAX-LLNA								
7	0.85	0.10	1.29	0.93	71.09	68.46	71.68	71.76
11	0.70	0.72	0.75	0.77	66.09	66.76	65.70	69.05
LLNA-CAN								
8	0.60	0.68	0.68	0.71	65.98	70.93	71.39	69.45
12	0.93	0.98	0.86	0.94	67.63	67.43	66.35	66.22
LLNA-FLAX								
5	0.81	1.08	0.83	0.92	69.11	70.69	69.00	66.59
6	0.51	0.55	0.58	0.59	64.69	65.69	65.14	67.15
CAN-LLNA								
2	0.65	0.64	0.59	0.64	66.05	66.90	65.64	68.17
4	0.78	0.76	0.77	0.67	69.05	70.14	70.69	68.84
CAN-FLAX								
9	0.98	0.96	0.99	0.83	70.52	71.22	72.96	72.00
10	0.90	0.95	1.06	1.07	68.92	65.51	67.36	67.50

Appendix 16 (cont'd).

Diet Order	Fatty Acid (% of total)							
	20:5				22:3			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	0.56	0.97	1.16	0.56	1.30	0.77	0.96	0.84
3	1.04	1.32	0.93	1.05	1.10	0.77	1.01	0.70
FLAX-LLNA								
7	0.64	1.38	0.69	0.58	0.90	0.46	0.80	0.57
11	0.54	1.15	0.69	0.74	0.89	0.55	0.87	0.63
LLNA-CAN								
8	0.58	0.59	0.47	0.76	0.67	0.48	0.31	0.38
12	0.78	0.62	0.82	0.86	0.61	0.47	0.61	0.44
LLNA-FLAX								
5	0.79	0.62	0.65	1.19	0.83	0.63	0.80	0.49
6	0.75	0.60	0.61	1.20	0.88	0.78	1.85	0.00
CAN-LLNA								
2	1.34	1.58	0.72	0.80	0.80	0.53	0.76	0.58
4	1.25	1.26	0.97	0.99	0.44	0.33	0.23	0.53
CAN-FLAX								
9	0.93	1.16	0.75	1.02	0.55	0.49	0.61	0.57
10	0.72	0.87	0.63	1.23	0.60	0.56	0.75	0.48

Appendix 16 (cont'd).

Diet Order	Fatty Acid (% of total)							
	22:4				22:5			
Subject Day:	8	26	44	62	8	26	44	62
FLAX-CAN								
1	11.50	9.81	10.69	10.43	8.73	9.27	8.88	7.65
3	14.10	13.07	14.07	13.38	7.91	8.58	8.44	7.62
FLAX-LLNA								
7	14.98	13.46	12.00	13.19	7.48	9.28	6.73	5.26
11	12.60	10.39	13.01	10.32	9.70	10.51	11.02	7.19
LLNA-CAN								
8	16.67	14.52	11.47	13.61	6.75	5.11	4.58	6.40
12	12.54	12.33	14.32	13.27	6.79	5.33	7.39	6.98
LLNA-FLAX								
5	13.42	12.82	12.46	11.89	7.09	5.19	6.12	7.73
6	15.41	14.85	14.79	11.59	8.28	6.64	6.79	8.58
CAN-LLNA								
2	10.50	10.55	13.54	13.03	8.98	7.67	9.53	7.62
4	10.22	9.88	8.97	12.61	7.74	6.69	5.99	7.58
CAN-FLAX								
9	9.14	9.38	9.85	8.60	7.63	7.20	7.83	7.98
10	12.92	12.27	15.46	11.33	7.58	7.28	8.74	8.49

Appendix 16 (cont'd).

Diet Order / Subject	Fatty Acid (% of total) 22:6			
Day:	8	26	44	62
FLAX-CAN				
1	4.92	4.53	4.44	3.87
3	5.49	4.71	5.20	4.80
FLAX-LLNA				
7	2.62	3.14	2.57	2.03
11	4.05	3.39	3.74	2.89
LLNA-CAN				
8	3.63	2.92	1.83	2.39
12	3.82	3.82	3.70	3.99
LLNA-FLAX				
5	4.69	3.92	3.23	3.70
6	3.95	3.92	3.20	3.54
CAN-LLNA				
2	6.91	5.55	5.21	4.65
4	4.23	3.01	2.30	3.12
CAN-FLAX				
9	4.68	4.27	3.53	3.31
10	3.02	2.83	3.05	2.26

NOTE: 20:2 was not present in platelet PPE.

Appendix 17. t-Test Contrasts for the Changes in Plasma Total and Lipoprotein Cholesterol Levels on the Experimental Diets

Plasma Lipid (mmol/L)	MF vs. LLNA		MF vs. CAN		MF vs. FLAX	
	t-value	prob	t-value	prob	t-value	prob
TC	-6.58 †	0.00	-7.30 †	0.00	-9.39 †	0.00
LDL-C	-6.58 †	0.00	-5.65 †	0.00	-7.77 †	0.00
VLDL-C	-1.12	0.29	-3.11 †	0.01	-2.02	0.07
HDL-C	0.48	0.64	-0.97	0.36	-2.59 †	0.03
TG	-6.58 †	0.10	-7.30	0.13	-9.39 †	0.05

† Significantly different ($p<0.05$) from the MF diet.

Appendix 18. t-Test Contrasts for the Comparison of the Changes in Plasma Total and Lipoprotein Cholesterol Levels Among the Experimental Diets[1]

Plasma Lipid (mmol/L)	LLNA vs. CAN		LLNA vs. FLAX	
	t-value	prob	t-value	prob
TC	0.51	0.62	1.99	0.08
LDL	-0.66	0.53	0.85	0.42
VLDL	1.41	0.19	0.64	0.54
HDL	1.02	0.33	2.17	0.06
TG	-0.09	0.93	0.33	0.75

[1] Plasma total and lipoprotein cholesterol levels did not significantly differ ($p<0.05$) among the experimental diets.

Appendix 19. t-Test Contrasts for the Changes in Platelet Phosphatidylcholine Fatty Acid Levels on the Experimental Diets

Fatty Acid[1]	MF vs. LLNA		MF vs. CAN		MF vs. FLAX	
	t-value	prob	t-value	prob	t-value	prob
16:0	0.78	0.46	0.74	0.48	0.35	0.73
18:0	-7.75 †	0.00	-6.92 †	0.00	-6.32 †	0.00
18:1	1.45	0.18	0.76	0.47	0.25	0.81
18:2	6.31 †	0.00	6.91 †	0.00	7.36 †	0.00
18:3	3.53 †	0.01	9.10 †	0.00	15.20 †	0.00
20:2	11.01 †	0.00	10.41 †	0.00	12.22 †	0.00
20:3	-6.47 †	0.00	-7.70 †	0.00	-8.46 †	0.00
20:4	-3.97 †	0.00	-4.93 †	0.00	-4.00 †	0.00
20:5	-1.54	0.16	-1.76	0.11	2.88 †	0.02
22:4	-1.35	0.21	-2.78 †	0.02	-2.01	0.08
22:5	-4.18 †	0.00	-3.58 †	0.01	0.97	0.36
22:6	-1.17	0.27	-3.26 †	0.01	-0.37	0.72
n-3 PUFA[2]	-2.52 †	0.03	-3.57 †	0.01	0.67	0.52
n-6 PUFA[3]	-4.39 †	0.00	-5.59 †	0.00	-4.77 †	0.00

[1] Carbon chain length:number of double bonds.
[2] n-3 = 20:5 + 22:5 + 22:6.
[3] n-6 = 20:3 + 20:4 + 22:4.
† Values are significantly different ($p<0.05$) from the MF diet.

Appendix 20. t-Test Contrasts for the Changes in Platelet Phosphatidylethanolamine Fatty Acid Levels on the Experimental Diets

Fatty Acid[1]	MF vs. LLNA		MF vs. CAN		MF vs. FLAX	
	t-value	prob	t-value	prob	t-value	prob
16:0	-1.75	0.11	-2.65 †	0.03	-1.54	0.16
18:0	-4.53 †	0.00	-4.02 †	0.00	-3.34 †	0.01
18:1	11.07 †	0.00	9.11 †	0.00	8.18 †	0.00
18:2	10.80 †	0.00	10.36 †	0.00	15.24 †	0.00
18:3	2.63 †	0.03	3.51 †	0.01	7.37 †	0.00
20:2	3.22 †	0.01	0.44	0.67	1.73	0.12
20:3	2.32 †	0.05	3.27 †	0.01	1.45	0.18
20:4	-7.10 †	0.00	-4.85 †	0.00	-6.08 †	0.00
20:5	-2.81 †	0.02	-0.42	0.69	4.94 †	0.00
22:4	-1.15	0.28	-1.02	0.34	-3.01 †	0.01
22:5	-6.32 †	0.00	-3.02 †	0.01	0.76	0.46
22:6	-1.92	0.09	-1.22	0.25	-0.55	0.60
n-3 PUFA[2]	-4.17 †	0.00	-2.13	0.06	0.37	0.72
n-6 PUFA[3]	-6.07 †	0.00	-4.17 †	0.00	-5.86 †	0.00

[1] Carbon chain length:number of double bonds.
[2] n-3 = 20:5 + 22:5 + 22:6.
[3] n-6 = 20:3 + 20:4 + 22:4.
† Values are significantly different ($p<0.05$) from the MF diet.

Appendix 21. t-Test Contrasts for the Changes in Platelet Alkenylacyl Phosphoglyceride Fatty Acid Levels on the Experimental Diets

Fatty Acid[1]	MF-LLNA		MF-CAN		MF-FLAX	
	t-value	prob	t-value	prob	t-value	prob
16:0	-0.99	0.35	-0.72	0.49	-2.73 †	0.02
18:0	-2.67 †	0.03	-3.25 †	0.01	-1.63	0.14
18:1	3.31 †	0.01	3.33 †	0.01	3.48 †	0.01
18:2	2.78 †	0.02	2.04	0.07	4.37 †	0.00
18:3	0.60	0.56	0.17	0.87	1.94	0.08
20:3	0.34	0.74	-0.24	0.82	-1.44	0.18
20:4	1.88	0.09	0.42	0.68	-0.25	0.81
20:5	-0.97	0.36	0.93	0.38	7.32 †	0.00
22:4	-0.21	0.84	-0.06	0.95	-1.69	0.12
22:5	-3.67 †	0.01	-1.12	0.29	2.13	0.06
22:6	-1.37	0.21	-1.72	0.12	-0.79	0.45
n-3 PUFA[2]	-2.90 †	0.02	1.24	0.25	1.82	0.10
n-6 PUFA[3]	1.31	0.22	-1.78	0.11	-1.60	0.14

[1] Carbon chain length: number of double bonds.
[2] n-3 = 20:5 + 22:5 + 22:6.
[3] n-6 = 20:3 + 20:4 + 22:4.
† Values are significantly different ($p<0.05$) from the MF diet.

Appendix 22. t-Test Contrasts for the Changes in Platelet Platelet Phosphatidylcholine Fatty Acid Levels Among the Experimental Diets

Fatty Acid[1]	LLNA vs. CAN		LLNA vs. FLAX	
	t-value	prob	t-value	prob
16:0	0.03	0.98	0.30	0.77
18:0	-0.23	0.82	-1.01	0.34
18:1	0.49	0.64	0.85	0.42
18:2	-0.42	0.68	-0.74	0.48
18:3	-3.94 †	0.00	-8.25 †	0.00
20:2	0.43	0.68	-0.27	0.79
20:3	0.87	0.41	1.41	0.19
20:4	0.68	0.51	0.02	0.98
20:5	0.15	0.88	-3.13 †	0.01
22:4	1.01	0.34	0.47	0.65
22:5	-0.43	0.68	-3.64 †	0.01
22:6	1.48	0.17	-0.56	0.59
n-3 PUFA[2]	0.74	0.48	-2.26 †	0.05
n-6 PUFA[3]	0.85	0.42	0.26	0.80

[1] Carbon chain length: number of double bonds.
[2] n-3 = 20:5 + 22:5 + 22:6.
[3] n-6 = 20:3 + 20:4 + 22:4.
† Values are significantly different ($p<0.05$) from the other experimental diet.

Appendix 23. t-Test Contrasts for the Comparison of the Changes in Platelet Phosphatidylethanolamine Fatty Acid Levels Among the Experimental Diets

Fatty Acid[1]	LLNA vs. CAN		LLNA vs. FLAX	
	t-value	prob	t-value	prob
16:0	0.63	0.54	-0.15	0.88
18:0	-0.36	0.73	-0.84	0.42
18:1	1.83	0.10	2.49 †	0.03
18:2	0.31	0.76	-3.14 †	0.01
18:3	-0.63	0.55	-3.35 †	0.01
20:2	1.97	0.08	1.05	0.32
20:3	-0.68	0.52	0.61	0.55
20:4	-1.59	0.15	-0.72	0.49
20:5	-1.69	0.13	-5.47 †	0.00
22:4	-0.10	0.92	1.32	0.22
22:5	-2.33 †	0.04	-5.01 †	0.00
22:6	-0.50	0.63	-0.97	0.36
n-3 PUFA[2]	-1.44	0.18	-3.20 †	0.01
n-6 PUFA[3]	-1.35	0.21	-0.15	0.88

[1] Carbon chain length:number of bonds.
[2] n-3 = 20:5 + 22:5 + 22:5.
[3] n-6 = 20:3 + 20:4 + 22:4.
† Values are significantly different ($p<0.05$) from the other experimental diet.

Appendix 24. t-Test Contrasts for the Comparison of the Changes in Platelet Alkenylacyl Phosphoglyceride Fatty Acid Levels Among the Experimental Diets

Fatty Acid[1]	LLNA vs. CAN		LLNA vs. FLAX	
	t-value	prob	t-value	prob
16:0	-0.19	0.85	1.23	0.25
18:0	0.41	0.69	-0.73	0.48
18:1	-0.02	0.99	-0.12	0.90
18:2	0.52	0.62	-1.12	0.29
18:3	0.30	0.77	-0.94	0.37
20:3	0.41	0.69	1.26	0.24
20:4	1.51	0.17	1.03	0.33
20:5	-1.34	0.21	-5.86 †	0.00
22:4	-0.10	0.92	1.05	0.32
22:5	-1.80	0.10	-4.10 †	0.00
22:6	0.24	0.81	-0.42	0.69
n-3 PUFA[2]	-1.10	0.30	-3.34 †	0.01
n-6 PUFA[3]	2.19	0.06	2.06	0.07

[1] Carbon chain length:number of double bonds.
[2] n-3 = 20:5 + 22:5 + 22:6.
[3] n-6 = 20:3 + 20:4 + 22:4.
† Values are significantly different ($p<0.05$) from the other experimental diet.