THE EFFECT OF DIETARY CANOLA OIL AND SUNFLOWER OIL ON PLASMA LIPIDS IN HEALTHY YOUNG MEN

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by

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

A 48-day metabolic study involving 8 normolipidemic men was divided into 4 diet periods: a 6 day pre-experimental, two-18 day experimental and a 6-day post experimental. Approximately 75% of the dietary fat (28% of total energy) was provided by a mixture of fats during the pre- and post-experimental periods and canola oil (CO) or sunflower oil (SO) during the experimental periods. The CO and SO diets were fed in a cross-over design. Saturated fatty acids provided 14, 5 and 7%, monounsaturated fatty acids 15, 20 and 7% and polyunsaturated fatty acids 7, 10 and 22% of total dietary energy in the mixed fat, CO and SO diets, respectively. The ratios of linoleic to linolenic acid were 2.6 to 1 and 73.9 to 1 in the CO and SO diets, respectively. Venous blood samples were taken at the beginning and end of each diet period from subjects who had fasted 12 hours. The CO and SO diets produced similar decreases in serum cholesterol (20 and 14%, respectively) and LDL-cholesterol (25 and 21%, respectively). Neither fat source affected plasma HDL-cholesterol or triglyceride levels. However, dietary fat source did have an effect on plasma phospholipid and cholesterol ester fatty acids: 18:1 n-9, 18:3 n-3 and 20:5 n-3 were significantly higher ($p < 0.05$) and 18:2 n-6 significantly lower in the phosphatidylcholine (PC) fraction, 18:1 was significantly higher and 20:4 significantly lower in the

phosphatidylethanolamine (PE) fraction, 18:1 and 20:5 were significantly higher and 20:4 and 22:6 were significantly lower in the lyso-PE fraction and 18:1, 18:3 and 20:5 were significantly higher and 18:2 significantly lower in cholesterol esters on the CO diet compared to the SO diet. Thus it would appear that the experimental diets had equal hypocholesterolemic effects and that consumption of the CO diet resulted in a higher n-3 fatty acid and lower n-6 fatty acid content in plasma phospholipids and cholesterol esters compared to the SO diet.

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LIST OF ABBREVIATIONS

FULL NAME	ABBREVIATION(S) USED
adenosine diphosphate	ADP
arachidonic acid	20:4, 20:4(n-6)
canola-sunflower group	CAN-SUN
carbohydrates	CHO
cholesterol esters	CE
coronary heart disease	CHD
docosahexaenoic acid	DHA, 22:6, 22:6(n-3)
eicosapentaenoic acid	EPA, 20:5, 20:5(n-3)
high density lipoprotein	HDL
linoleic acid	18:2, 18:2(n-6)
linolenic acid	18:3, 18:3(n-3)
low density lipoprotein	LDL
low erucic acid rapeseed oil	LEAR
lyso-phosphatidylethanolamine	LPE
monounsaturated fatty acids	MUFA
oleic acid	18:1, 18:1(n-9)
palmitic acid	16:0
phosphatidylcholine	PC
phosphatidylethanolamine	PE
polyunsaturated fatty acids	PUFA
saturated fatty acids	SFA
stearic acid	18:0

LIST OF ABBREVIATIONS continued

FULL NAME	ABBREVIATION(S) USED
sunflower-canola group	SUN-CAN
total cholesterol	TC
thin layer chromatography	TLC
triglycerides	TG
very low density lipoproteins	VLDL

REVIEW OF LITERATURE

Atherosclerosis refers to a type of hardening of the arteries involving the infiltration of fatty materials into the intima. Atherosclerotic lesions essentially result in a thickening of the intima and a decrease in the size of the lumen, the first stage of coronary heart disease (CHD).

The most widely accepted hypothesis of atherogenesis suggests that platelets play a role in the development of the disease (Ross, 1986 and Betteridge, 1987). If there is injury to the endothelial wall, the subendothelial connective tissue is exposed to platelets which adhere to collagen and then aggregate. These events are followed by focal proliferation of arterial smooth muscle cells, formation of connective tissue and deposition of lipids. Continuous or repeated injury to the endothelium results in an enlarged lesion. This type of lesion is most often associated with the occlusion of the arterial lumen, which may result in a myocardial infarction (MI), the second stage of CHD.

Coronary heart disease is a major health problem in the developed world. In Canada, it has been the leading cause of death for decades (Ku et al, 1987). The positive relationship between risk of CHD and plasma concentrations of total cholesterol (TC) and low density lipoprotein (LDL) cholesterol is well accepted, as is the positive

association between levels of TC and severity of atherosclerosis. Clinical studies indicate that diet affects serum lipid levels. Thus it is not surprising that the identification of the dietary components that will alter serum lipids and lipoproteins favourably has been a research priority for the past 30 years.

The Relationship between Dietary Fatty Acids and Plasma Lipids and Lipoproteins

Many of the current recommendations with respect to dietary fat and its effects on TC are based on the work of Keys and his associates (1957a) at the University of Minnesota. A series of controlled metabolic experiments by this group produced the following prediction equation that described the relationship between changes in dietary fat and the expected change in serum TC.

$$\Delta chol = -1.68 + 2.76 \Delta S + 0.05 \Delta M - 1.35 \Delta P$$

where,

- Δ chol = estimated average change in serum TC (within 2-4 weeks) in mg/100mL.
- ΔS = difference in percent of total energy from dietary saturated fatty acids (SFA).
- ΔP = difference in percent of total energy from dietary polyunsaturated fatty acids (PUFA).
- ΔM = difference in percent of total energy from dietary monounsaturated fatty acids (MUFA).

It was found that the contribution of change in percent of total energy from dietary MUFA to the change in TC was not significant and a simpler prediction equation

resulted.

$\triangle chol = 2.74 \triangle S - 1.31 \triangle P$

where, chol, $\triangle$ S and $\triangle$ P are as described previously (Keys et al.,1957a).

Despite its wide acceptance, the conclusion that MUFA have no effect in lowering TC was not fully supported by the work of Keys et al.(1957a). With the exception of olive oil (very high in MUFA), coconut oil (high in SFA) and safflower oil (high in PUFA) the nine test diets contained relatively consistent amounts of MUFA when compared to the variation in PUFA and SFA in the same diets. It is therefore possible that any effect of MUFA on TC could have been masked by the small variation in MUFA among diets. In addition, in approximately 70% of all diet comparisons a decrease or increase of SFA was accompanied by a concomitant decrease or increase in MUFA. If in fact MUFA have more than a passive effect on TC, this situation could have prevented its detection.

Subsequent work by Keys et al. (1965) demonstrated that lauric, myristic and palmitic acids had a hypercholesterolemic effect not shared by stearic acid and shorter chain SFA. Although it would appear inappropriate to generalize a hypercholesterolemic effect to all SFA there is little argument that certain dietary SFA raise TC. On the other hand there is controversy over what should replace this energy source.

Dietary PUFA, particularly linoleic acid, have received the majority of attention as a substitute for dietary SFA. Keys et al. (1957b) observed that when dietary linoleic acid was exchanged for SFA, TC decreased. However, Vega et al. (1982) and Shepherd et al. (1978) demonstrated a lowering effect of PUFA on high density lipoprotein (HDL) cholesterol; perhaps through an inhibition of the synthesis of apolipoprotein A-1, the major apolipoprotein of HDL (Shepherd et al. 1978). This effect is considered undesirable because of the inverse relationship between HDL-cholesterol and CHD (Castelli et al. 1986).

Concern among nutritionists and clinicians about the possible adverse effects of substituting dietary SFA with dietary PUFA has given rise to recent investigations of the relative influence of PUFA and MUFA on plasma lipids and lipoproteins. Recent work by Mattson and Grundy (1985) found that both oleic acid and linoleic acid resulted in similar reductions in TC and LDL cholesterol concentrations. Mattson and Grundy (1985) compared the effect of 3 formula diets comprised of 40% of total energy as fat, with the fatty acids being predominantly SFA, MUFA or PUFA. Of the 20 patients used in the study 12 had normal triglyceride (TG) levels, which were not affected by either the MUFA or PUFA diets. On the other hand, both the MUFA and PUFA diets lowered TC and both produced an equal

lowering of LDL-cholesterol in these subjects when compared to the SFA diet. HDL-cholesterol was not changed significantly by either diet. Responses to dietary fat source were variable among the remaining 8 (hypertriglyceridemic) subjects. Perhaps the most important finding of this study was the identical reduction in plasma LDL-cholesterol in the normo-triglyceridemic group fed the PUFA and MUFA diets. Mattson and Grundy (1985) noted the lack of agreement between their results and the findings of Keys et al. (1957a). They postulated that the greater reduction in TC produced by PUFA compared to MUFA observed by Keys et al. may have been due to a greater decrement in the HDL-lipoprotein fraction.

Subsequent studies also suggested that MUFA and PUFA have comparable hypocholesterolemic effects. Lasserre et al. (1985) examined the effect of 4 test fats on TC and TG. The diets provided 30% of total energy as fat, 54% as carbohydrate (CHO) and 16% as protein. Two thirds of the fat was made up of sunflower, peanut or low erucic acid rapeseed (LEAR) oil or milk fats. As a consequence the diets varied appreciably in oleic, linoleic and linolenic acids. The milk fat diet resulted in the highest observed mean TC (243 mg/100 mL), while the lowest was observed following the sunflower oil period (175 mg/100 mL). Mean TC following the LEAR oil diet (190 mg/100 mL) was significantly lower than following the milk fat diet and

did not differ ($p > 0.05$) from the level observed following the sunflower oil regimen. The peanut oil diet, despite containing similar amounts of MUFA and PUFA to the LEAR oil diet resulted a mean TC (205 mg/100 mL) that was significantly higher than that of the sunflower oil period. Interestingly, the structure of the component triglycerides in peanut oil appear to be, in part, responsible for the unique behaviour of this oil (Kritchevsky, 1984). Although TG were lowest after consumption of the LEAR oil diet, no significant differences were observed following diet periods.

Sirtori et al. (1986) compared the effects of olive oil (oleic acid-rich) and corn oil (linoleic acid-rich) on plasma lipids and lipoproteins of subjects at risk for CHD. The diets were fed in a cross-over design and contained 30% of total energy as fat, with MUFA providing 36% and 9% and PUFA 36% and 64% of total fat in the corn oil and olive oil diets, respectively. In individuals consuming the corn oil diet, followed by the olive oil diet, consumption of the corn oil diet resulted in a significant decrease in TC while switching to the olive oil diet produced no further changes. In those individuals consuming the olive oil diet followed by the corn oil diet, consumption of the olive oil diet did not result in any significant changes in TC whereas switching to the corn oil diet resulted in a significant decrease in TC. Changes in TC could primarily

cholesterol levels. The ratio of LDL-cholesterol to HDL-cholesterol was significantly lower during the MUFA-rich diet compared to the ratio during the CHO-rich diet. Another study of the effects of MUFA versus complex CHO on plasma cholesterol (Mensink and Katan, 1987) supported many of the above findings. After consuming a SFA-rich diet (38% of total energy as fat, 48% as CHO) matched pairs of subjects received either a high CHO (22% of total energy as fat, 62% as CHO) or a MUFA-rich olive oil diet (41% of energy as fat, 46% as CHO). Both diets resulted in similar decreases in plasma TC when compared to the SFA diet. Triglycerides increased with the CHO-rich diet and decreased with the MUFA-rich diet. Conversely, HDL-cholesterol decreased significantly following the CHO-rich diet and did not change following the MUFA diet. Overall, the MUFA diet produced more favourable changes in plasma lipid and lipoprotein levels than the low fat regimen. In a similar study, Baggio et al. (1988) compared the effects of a MUFA-rich olive oil diet (38 and 46% of total energy from fat and CHO, respectively) to a CHO-rich diet (28 and 56% of total energy from fat and CHO, respectively). Mean TC, LDL-cholesterol, TG and total apolipoprotein B levels were significantly lower following the MUFA-rich diet compared to the levels observed following the CHO-rich diet. Unlike the studies by Grundy (1986b) and Mensink and Katan (1987) there were no differences in mean HDL-

be accounted for by changes in LDL cholesterol, however, HDL-cholesterol also decreased following the corn oil diet. Thus the LDL to HDL cholesterol ratios were the same for both diets; this ratio has been accepted as a reliable index of atherogenic risk (Gordon et al. 1981). Therefore, the olive oil diet was considered comparable to the corn oil diet in effecting changes in plasma lipoproteins. What is of interest is, that this occurred at a lower total fat intake than in the Mattson and Grundy (1985) study.

The results of these three studies support the findings that canola oil (approximately 60% oleic acid) is effective in lowering TC levels of young men (McDonald, 1983). Similarly, results from studies comparing the effects of dietary MUFA and dietary fat reduction on plasma lipids and lipoproteins demonstrate the hypocholesterolemic effect of oleic acid. Grundy (1986b) conducted a metabolic study comparing liquid formula diets rich in either SFA or MUFA (each containing 40% of total energy as fat and 43% of total energy as CHO) to a CHO-rich formula diet (20% of total energy as fat and 63% of total energy as CHO). The MUFA and CHO-rich diets lowered TC and LDL-cholesterol levels, compared to the SFA-rich diet, by 13 and 7% and by 21 and 15%, respectively. The MUFA regimen had no effect on TG and produced a non-significant decrease in HDL-cholesterol. The CHO-rich diet produced a non-significant increase in TG and a significant decrease in HDL-

cholesterol levels between the two diets.

The results of these studies indicated that diets rich in MUFA, unlike low fat (CHO-rich) diets, caused a specific lowering of LDL-cholesterol without increasing TG levels. Futhermore, these results suggest that a reduction of total dietary fat may not be the most effective strategy in the prevention of CHD.

Diets rich in MUFA do not appear to have the health concerns associated with those rich in PUFA. Their consumption in the Mediterranean region over centuries indicates that they are a relatively safe dietary fat source. Despite consumption of a fairly high fat diet, this region has a particularly low incidence of CHD (Keys, 1970), which may, in part, be attributed to a high intake of oleic acid. Interestingly, "westernization" of the diet in affluent areas of Italy has resulted in a concomitant increase in serum lipid levels. People in the rural regions of Italy continue to consume the conventional Mediterranean diet, characterized by relatively high levels of oleic acid and complex CHO. Ferro-Luzzi et al. (1984) have shown experimentally that conversion from the traditional Italian diet to a "western" diet results in unfavourable plasma lipid changes. They recruited subjects from rural southern Italy and "westernized" their diets, primarily through the manipulation of dietary fat (increased from 33 to 37% of total energy) and CHO

(decreased from 45 to 40% of total energy). The traditional diet contained 17, 9-11, and 4% of total energy from oleic acid, SFA and PUFA, respectively, while the modified diets contained 15-16, 15-17 and 13-14% of total energy from these respective fatty acids. These dietary manipulations resulted in an increase in TC of 15-16%, an increase in LDL-cholesterol of 19% and an increase in HDL-cholesterol of 0-19%. The results of this study are of particular interest considering the modest nature of the dietary changes.

The n-3 family of fatty acids, including linolenic acid, appear to have different effects on plasma lipoprotein metabolism than those of the n-6 family, such as linoleic acid. Jacotot et al. (1986) investigated the effect of dietary linoleic acid with or without dietary linolenic acid on lipoprotein metabolism. A long term metabolic study compared the effects of three dietary regimens, all containing 30% of total energy from fat. A sunflower oil diet (71% of fat from linoleic acid) and a corn oil diet (61% of fat from linoleic acid) were compared to a soybean oil diet (51% of fat from linoleic acid and 12% from linolenic acid). Total cholesterol was significantly lower after consuming the sunflower oil diet as compared to the other diets. Interestingly, HDL-cholesterol levels were highest after consumption of the soybean oil diet and were significantly lower after the

corn oil period. Triglyceride levels remained unchanged. These results suggest that the ratio between dietary linoleic and linolenic acids may influence plasma lipid and lipoprotein levels. However, the relative importance and the optimum intakes of these fatty acids is not known.

In other studies, the longer chain n-3 fatty acids from fish oils have been shown to have TG lowering effects which are not shared by oils rich in linoleic and linolenic acid. Three to 5 g/day of eicosapentaenoic acid (EPA) lowered TG in patients with hypertriglyceridemia (Sanders et al. 1985), perhaps through the inhibition of formation of very low density lipoproteins (VLDL) by the liver. In a similar study, 15 to 20 g/day of EPA resulted in a reduced rate of VLDL, TG and apolipoprotein B production (the major apolipoprotein of LDL) by the liver (Nestel et al. 1984). It was concluded that EPA did not have a unique role in the treatment of hypercholesterolemia, other than in the treatment of conditions of excess VLDL cholesterol (Nestel, 1987). However, it should be noted that n-3 fatty acids have other physiological effects which are important in the etiology of CHD. These effects will be discussed in the following section.

The Relationship between Dietary Fatty Acids and Prostanoid Synthesis

The recognition of thrombogenesis as an important process in CHD has led to investigation of the effects of

dietary fatty acids on lipid metabolism and prostanoid synthesis in platelet and endothelial cells. Although it is not fully understood, the basic mechanism of thrombosis includes: fibrin coagulation, platelet adhesion, platelet aggregation and decreased fibrinolysis, all of which appear to be initiated by injury to the endothelial lining of an artery wall (Mustard and Packham, 1970). Thus, changes in the interactions between platelets and endothelial cells through diet-induced changes in lipid metabolism may promote or inhibit thrombogenesis and thus may promote or inhibit the development and complications of CHD.

The body can use three different fatty acids as precursors of prostanoids, di-homo- γ -linolenic acid, arachidonic acid and EPA which give rise to series 1, 2 and 3 prostanoids, respectively. However, because di-homo-γ-linolenic acid is thought to be easily converted to arachidonic acid, biosynthesis of series 1 and 2 prostaglandins are often considered together. This approach, however, may not be appropriate as there is some suggestion that humans may convert only limited amounts of di-homo-γ -linolenic acid to arachidonic acid (Dorfman, 1985).

Prostanoids of the three series do not always have the same effects, thus dietary concentrations of precursor fatty acids are not only relevant in determining the eventual prostaglandin type, but also the physiological

action. For example, thromboxane A_2 is a potent platelet aggregator, while thromboxane A_3 (produced from EPA) is a relatively weak platelet aggregator. By contrast, both prostacyclins 2 and 3 (PGI_2 and PGI_3) have similar platelet anti-aggregator activities.

When platelets and endothelial cells are stimulated by specific agents, such as adenosine diphosphate, epinephrine, collagen and thrombin (Longnecker, 1982) they synthesize prostanoids through the pathway described in Figure 1. It is generally accepted that platelets have a propensity to produce thromboxane A_2 while endothelial cells have a propensity to produce prostacyclin (Kirkland et al., 1986). These compounds have strong effects on the interactions between these two cell types.

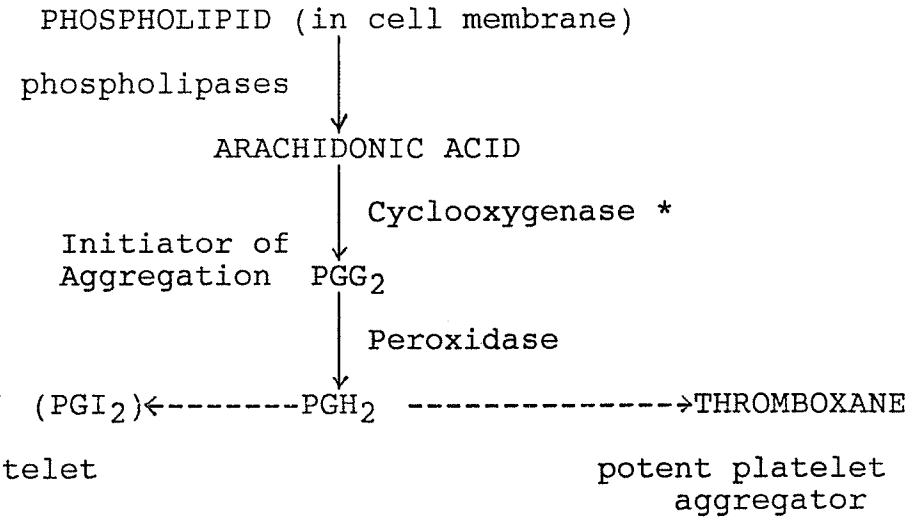


Figure 1 Pathways of synthesis of series 2 prostacyclin and thromboxane from arachidonic acid in platelets and endothelial cells (Smith and Borgeat, 1985). * platelet cyclooxygenase is thought to be acetylated and therefore rendered inactive by aspirin (Huang et al., 1986).

Recently, fatty acids of the n-3 family have received considerable attention because of their effects on lipid and prostaglandin metabolism. Evidence of an inverse relationship between amounts of dietary n-3 fatty acids with greater than 18 carbons and platelet function is accumulating. A decrease in platelet-vessel wall interactions and platelet aggregation has been observed following the consumption of EPA (Dyerburg, 1981). Wiener et al. (1986) found that dietary EPA resulted in reduced platelet arachidonic acid, increased platelet EPA and decreased serum thromboxane levels in swine. Interestingly, despite elevated serum lipids, the development of atherosclerosis in these animals was retarded, perhaps through altered prostaglandin metabolism. This effect appears to be associated with significant increases in the level of EPA in individual platelet phospholipids and a corresponding decrease in arachidonic acid (Herold and Kinsella, 1986).

There is some suggestion that 20 and 22 carbon n-3 fatty acids have to be supplied directly from the diet to induce their biological effects in humans. However, α-linolenic acid can be chain elongated and desaturated by humans; via the pathway common to all unsaturated fatty acids (Figure 2). Linolenic acid is the preferred substrate for Δ 6-desaturase (Cook, 1985) and EPA, docosahexaenoic acid (DHA) (Leaf and Weber, 1988) and

linolenic acid (Hwang and Carroll,1980) inhibit the conversion of linoleic acid to arachidonic acid. Despite the general acceptance of this metabolic pathway, humans do not convert linolenic acid to EPA to any great extent (Dyerburg, 1986). Therefore, there is controversy surrounding the relative significance of linolenic acid on prostanoid metabolism.

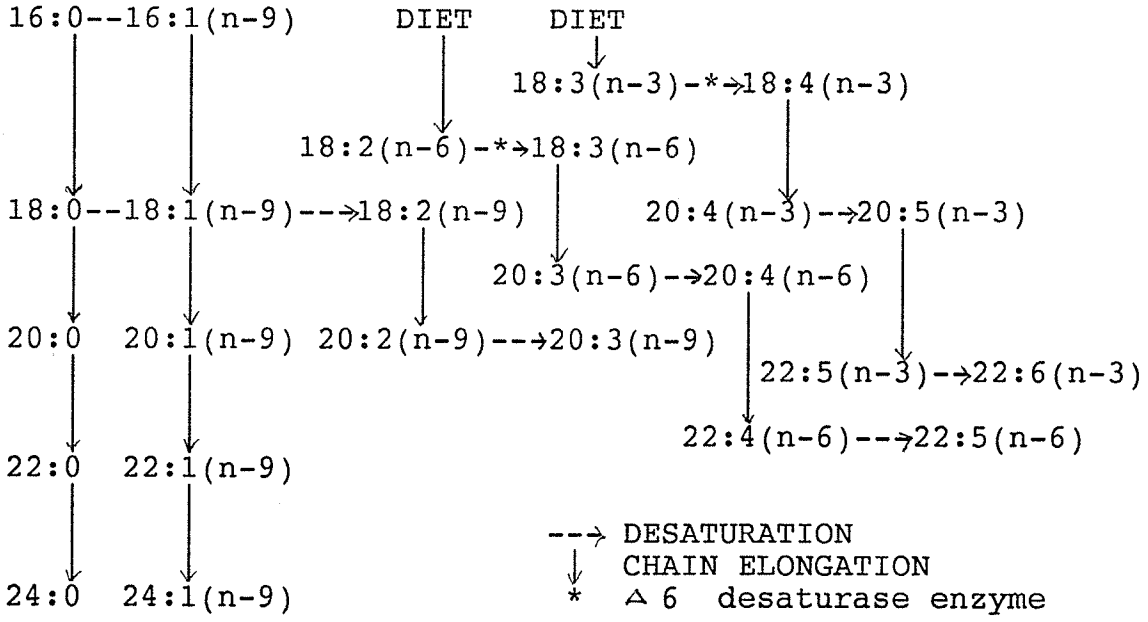


Figure 2. Major pathways of fatty acid biosynthesis by chain elongation and desaturation in animal tissues (Cook, 1985).

Incorporation of Dietary Fatty Acids and their Metabolites into Platelet and Plasma Phospholipids

Dyerburg (1986) compared a diet providing 25.5 g/day of linolenic acid from linseed oil to a diet providing 4.3 g/day of EPA from cod liver oil in a healthy volunteer. The diet periods were 8 days in duration. Following the

linseed oil diet the EPA content of the plasma phospholipids and cholesterol esters (CE) increased 0.7% and 0.5%, respectively. Following the cod liver oil diet, EPA in the phospholipids and CE each increased 6.4%. Dyerburg concluded that dietary linolenic acid was not an effective means of increasing the EPA content of plasma lipids. However, the use of only one subject and the lack of information on diet control make these results difficult to interpret.

Renaud and Norday (1983) acknowledged the moderate conversion of linolenic acid to EPA. However, they felt that these minor changes were significant in terms of the resulting changes in platelet behaviour. They supported this conclusion by the results of their study on the effects of diet modification on platelet function and composition (Renaud et al. 1986a). Four groups of men were studied over a period of 3 years. In the first year, one group received a margarine containing sunflower oil (53.7% PUFA) and another received a margarine containing both sunflower oil and LEAR oil (50.2% PUFA), both of these groups received an oil mixture of sunflower and LEAR oil. The other 2 groups served as controls. Those groups with dietary changes experienced a decrease in the clotting activity of washed platelets and a decreased response to thrombin induced aggregation. On the other hand, there was an increased response to ADP-induced aggregation. After 2

years the diets of the control groups also were modified: one control group received LEAR oil which provided both linoleic and linolenic acid; and the other received sunflower oil, which provided only linoleic acid. The diets of the original experimental groups also were changed at the 2 year mark. The PUFA content of the margarine was reduced to 26-38% of the fatty acids. Correlation coefficients were calculated using data collected from these various treatment groups, over a three year period. This analysis suggested that decreased clotting activity of washed platelets and decreased aggregation of platelets to thrombin were more closely associated with dietary linolenic acid than linoleic acid. Similarly, when there was a dietary source of linolenic acid, EPA was significantly increased in both plasma and platelet lipids. Unfortunately this study did not attempt to assess dietary concentrations of individual fatty acids other than linoleic acid and linolenic acid; a common criticism of studies designed to explain the biological effects of linolenic acid.

The work of Kromhout et al. (1985) emphasized the importance of assessing dietary EPA when studying the effects of lipids on platelet prostanoid metabolism. Data of food intake, TC, and other known CHD risk factors were collected from 852 Dutch men. After a 20 year follow-up period, age and dietary cholesterol were positively

associated with death from CHD while fish consumption was negatively correlated with death from CHD. Furthermore, even relatively low intakes of fish (1-2 times weekly) appeared to have protective effects against the development of CHD. Although a comprehensive biological explanation for the negative relationship between fish consumption and CHD was not offered, it was suggested that EPA provided from fish and, perhaps, other constituents of this food reduced platelet aggregation and increased bleeding time.

Many of the studies on the metabolism of PUFA, like those of Dyerburg (1986) and Renaud et al. (1986a) are confounded by methodology. For example, fatty acid composition is usually determined for total plasma phospholipid as opposed to individual phospholipid species. Fatty acid composition varies among different phospholipid species. Similarly, the concentrations of phospholipid species vary among tissues. It is therefore possible that subtle but potentially significant changes are masked when phospholipid species are not analyzed separately. This point was demonstrated by Weaver and Holub (1985) who found that washed human platelets selectively incorporated EPA into phosphatidylcholine (PC) and phosphtidylethanolamine (PE) while arachidonic acid was selectively incorporated into phosphatidylinositol. Similarly, Holub (1987) noted that the levels of EPA and DHA in the 2 position of the alkenylacyl species of PE and the alkylacyl species of PC

were significantly greater than the levels of these fatty acids in the diacyl species of these phospholipids.

Renaud et al. (1986b) conducted a study similar to that described previously (Renaud et al., 1986a). However, there was no dietary intervention. Dietary information was collected by a 24 hour recall, a 1-day weighed diet and chemical analysis of aliquots from 1 day menus. Linolenic acid was inversely related to thrombin-induced platelet aggregation, supporting previously reported results (Renaud et al. 1986a). In both of the Renaud studies, intake of SFA was the parameter most highly correlated with platelet aggregation to thrombin and clotting activity of washed platelets.

Results from a study on the effects of dietary linolenic acid on the fatty acid composition of platelets and plasma PC in vegan and omnivor subjects also suggested that linolenic acid is converted to EPA by the human (Sanders and Younger, 1981). Subjects consumed 20 g of linseed oil daily for 2 weeks. The mean daily fatty acid intake of the vegan diet was 16, 28 and 2 g of SFA, linoleic and linolenic acids, respectively, while the mean intake for the omnivor diet were 38, 7 and 1.3 g, respectively. The ratios of linoleic to linolenic acid in the basal diets were 16 and 6 for the vegans and omnivors, respectively. The addition of 6.5 g of linolenic acid saw this ratio decrease to 3 in the vegans and 1 in the

omnivors. Such ratios led to a two-fold increase in the EPA content of both plasma and platelet PC in the omnivors and a three-fold increase in the EPA content of plasma PC in the vegans. On the other hand, platelet PC EPA content did not change in the vegans. It was postulated that the adipose tissue of the vegans may have contained a large amount of linoleic acid which may have buffered any changes in response to changes in dietary linoleic to linolenic acid ratio.

Subsequent studies also support the conversion of linolenic acid to EPA by humans. Lasserre et al. (1985) compared the effects of dietary sunflower oil, peanut oil, LEAR oil and milk fat on the fatty acid composition of serum phospholipids and CE. Subjects consumed diets containing, 30% of total energy as fat. The SFA content of the sunflower, peanut and LEAR oil diets was similar (7.8-8.3 % of total energy) while the SFA content of the milk fat diet was relatively high (22.5% of total energy). Ratios of linoleic to linolenic acid were 105:1, 50:1, 3:1 and 0.6:1 in the sunflower, peanut, LEAR and milk fat diets, respectively. Linoleic acid was highest in serum phospholipids, after the sunflower oil diet and lowest after the milk fat diet, while the reverse was seen for dihomo- -linolenic acid and arachidonic acid. The LEAR and peanut oil diets resulted in similar proportions of the n-6 family fatty acids in the total phospholipid fatty acid

pattern. Linolenic acid, EPA and DHA were in highest proportion after the LEAR oil diet and lowest after the sunflower and peanut oil regimens. Docosapentaenoic acid was slightly lower after the sunflower oil regimen. The sunflower oil diet also resulted in significantly higher levels of stearic and myristic acid compared to the other test diets. Similar differences in the fatty acid composition in relation to dietary fat source were observed for the CE.

The Lasserre group (1985) concluded that the significantly higher levels of linolenic acid, EPA and DHA in serum phospholipid after consuming the LEAR oil diet could be of some benefit in the prevention of CHD. They suggested that because this diet did not cause a decrease in linoleic acid derivatives in the plasma phospholipid, there was no competition between the linolenic and linoleic acid for 6 desaturase at the intakes provided by this regimen. This observation is of interest in view of the earlier suggestion, that linolenic acid inhibits the conversion of linoleic acid to arachidonic acid.

Jacotot et al. (1986) examined the effect of diets varying in linoleic acid and linolenic acid content on the concentrations of prostanoid precursors in plasma phospholipids using three diets, all containing 30% of total energy from fat. A sunflower oil diet (71% of fat from linoleic acid) and a corn oil diet (61% of fat from

linoleic acid) were compared to a soybean oil diet (51% of fat from linoleic acid and 12% from linolenic acid). Following the consumption of the sunflower and corn oil diets levels of plasma phospholipid 20:3 n-6, 20:4 n-6, 20:5 n-3 and 22:6 n-3 were lower than those observed after the soybean oil diet. The implication is that the intake level of 3.2 g/day of linolenic acid (provided by the soybean oil diet) could have an effect on prostanoid metabolism and perhaps be of some benefit in the prevention of thrombosis.

Despite the findings of the two previous studies, Budowski and Crawford (1985) pointed to several animal studies as well as the Renaud work (1986a, 1986b) to suggest that linolenic acid and its metabolites are important in the human diet as regulators of arachidonic acid metabolism. They further suggested that identification of the significance of the ratio between dietary linoleic and linolenic acid is important to the understanding of the relationship between dietary fats and prostanoid metabolism.

There is some evidence that the desaturases and fatty acids are part of an intricate feedback mechanism controlling prostanoid metabolism (Dorfman, 1985). It has been suggested that EPA inhibits formation of thromboxane A_2 and PGI_2 formation from arachidonic acid in platelets and leads to the formation of thromboxane A_3 and PGI_3

(Dorfman, 1985). Similarly, it has been shown that linolenic acid inhibits prostaglandin formation from arachidonic acid (Zollner, 1986). This phenomenon has been demonstrated in rats fed the following ratios of linolenic to linoleic acid; 1/32, 1/7, 1/1 and 3.5/1 (Marshall and Johnston, 1982). As dietary linolenic acid increased the metabolites of linoleic acid in the liver and spleen, particularly arachidonic acid, decreased while n-3 metabolites increased. At 2 months, there was a significant decrease in prostanoid synthesis by the liver and an indication of a decrease in prostanoid synthesis in the spleen. These results were explained by effective competition by linolenic acid over linoleic acid for the desaturase enzyme and inhibition of cyclooxygenase by linolenic acid and EPA. Although platelets were not among the cell types studied by Marshall and Johnston, Holub (1987) suggested that EPA may reduce thromboxane A_2 formation in platelets through the inhibition of cyclooxygenase.

Zollner et al. (1979) reported a direct relationship between urinary prostanoid metabolites and dietary linoleic acid when 0, 10 and 50 g/day of dietary linoleic acid were compared. This relationship suggested that prostanoid biosynthesis in humans is enhanced by linoleic acid intake. By contrast, Ferretti et al. (1985) did not observe any changes in urinary prostaglandin E metabolites when dietary

linoleic acid was increased from 10 to 30 g/day. These results suggest that the relative effects of more modest changes in dietary linoleic acid may not overcome all of the endogenous factors influencing prostanoid metabolism.

Adam et al. (1986) described a dose related suppression of prostanoid biosynthesis, measured through urinary metabolites in response to the ingestion of linolenic acid. A decrease in prostanoid synthesis occurred in the absence of a decrease in the availability of arachidonic acid. Therefore, these authors suggested that this reduction was a result of inhibition of cyclooxygenase by linolenic acid.

In addition to influencing prostaglandin metabolism dietary fatty acids have inhibitory effects on the aggregatory activity of platelets, probably through changes in platelet phospholipid fatty acids (Renaud et al. 1987). This is important in that evidence is accumulating that suggests membrane fluidity plays an important role in the regulation of platelet aggregation. Hornstra and Rand (1986) noted that dietary sunflower oil (50% of energy) was associated with more fluid platelet membranes compared with platelets from rats fed a low fat control diet (5% of energy as fat). There is also evidence that EPA and DHA in the phospholipids may affect the physical properties of cell membranes and the function of membrane bound proteins (Leaf and Weber, 1988). For example, arachidonic acid and

EPA appear to be released more rapidly than DHA from platelet phospholipids by phospholipases (Croset et al., 1986). Thus DHA may inhibit platelet aggregation by means other than a direct effect on prostanoid metabolism.

SUMMARY

The hypercholesterolemic effect of certain SFA is well established. Although substitution of SFA by PUFA is effective in lowering TC and LDL-cholesterol levels, a lowering of HDL-cholesterol also has been reported. Substitution of SFA with MUFA appears to be as effective as substitution with PUFA in lowering TC and LDL-cholesterol, without affecting HDL-cholesterol. This may, in part, explain the lower incidence of CHD in populations consuming large amounts of oleic acid as part of a relatively high fat diet. Diets in which dietary CHO replaces SFA appear to increase plasma TG levels and to have a hypocholesterolemic effect that is not specific to any lipoprotein fraction. Optimal intakes of fatty acid classes for achieving and/or maintaining healthy levels of plasma lipids and lipoproteins are unknown as are the optimal ratios of individual fatty acids. Interestingly, the recent Canadian Consensus Conference on Cholesterol recommended the following principles of dietary modification for individuals with elevated blood lipids:

1) total dietary fat intake should not exceed 30% of total energy intake,
2) dietary SFA, particularly lauric, myristic and

palmitic acids, should not exceed 10% of total energy intake,

3) restrict intake of foods high in cholesterol,

4) dietary PUFA should not exceed 10% of total energy intake

5) dietary protein should be in the range of 10-15% of total energy intake and,

6) dietary CHO to make up remainder (55-60%) of total energy intake, emphasizing polysaccharides from foods containing dietary fiber

(Canadian Consensus Conference on Cholesterol:Final Report,1988).

These recommendations reflect conventional thinking with respect to the relationship between blood lipids and dietary fats and emphasize the substitution of dietary SFA with dietary CHO. Furthermore there is no mention of MUFA as having a potential role in the reduction of blood lipids.

Both dietary n-3 and n-6 fatty acids influence platelet aggregation and production of prostanoids by both platelets and the endothelium of the artery. Hirai et al (1987) proposed a mechanism for the aggregatory effects of n-3 fatty acids which has been adapted here (Figure 3).

The relative benefits between the common n-3 fatty acids found in foods, linolenic acid, EPA and DHA are not known, nor are the differences in their biological properties. Similarly, the optimum ratios among dietary n-3, n-6 and other fatty acids and the possible hazards associated with increased use of n-3 fatty acids are not known. Hornstra et al., (1979) caution against a premature recommendation for a widespread use of n-3 fatty acids. They note that feeding n-3 fatty acids results in major

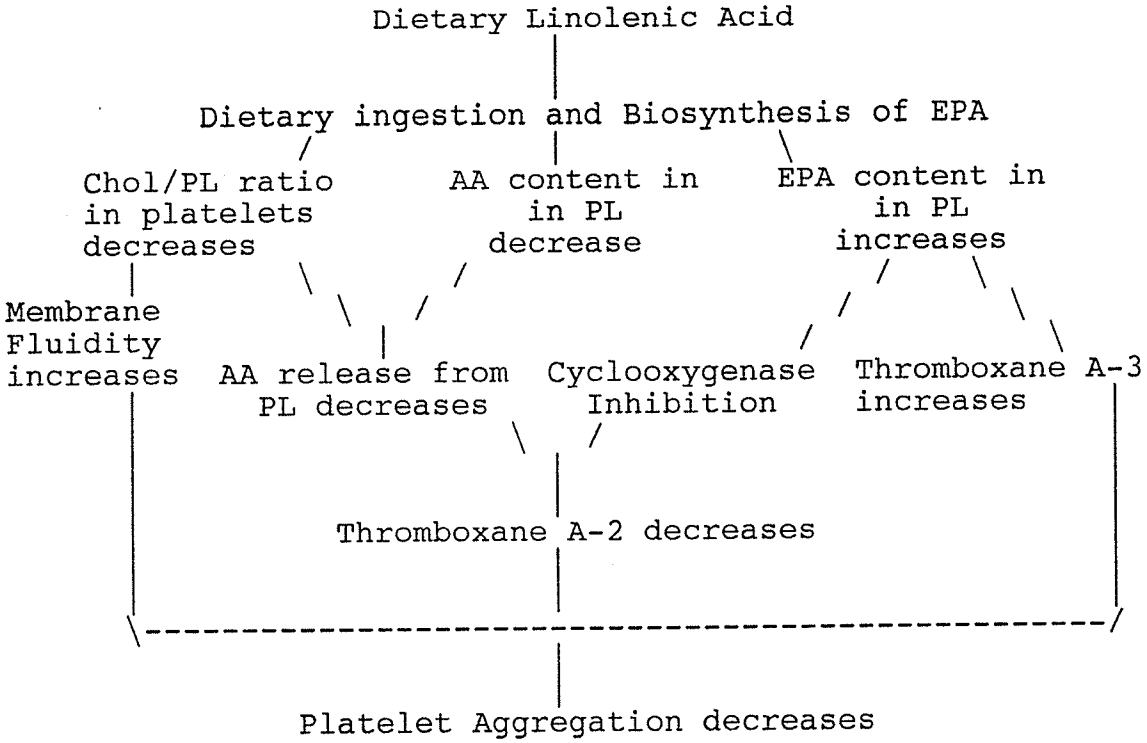


Figure 3. A proposed mechanism of anti-aggregatory effect of dietary n-3 fatty acids, where Chol: cholesterol, PL: phospholipids and AA: arachidonic acid.

changes in the composition of cardiac lipids which may have undesirable consequences. Furthermore, researchers generally emphasize the harmful effects of prostanoids in relation to thrombosis, however their beneficial effects in relation to wound healing can not be ignored. Therefore, the importance of understanding the control of prostanoid mobilization, and its normal and preferred states of activation can not be understated (Lands, 1986).

OBJECTIVES OF THE STUDY

The primary objectives of the present study were to investigate the effects of canola oil and sunflower oil as major sources of dietary fat on plasma lipid and lipoprotein patterns and the fatty acid composition of plasma phospholipids and cholesterol esters in healthy young men, when these fats provided 28% of total energy in a mixed diet.

Canola oil was used in this study because of its rather unique fatty acid composition compared to other vegetable oils. Canola oil is characterized by a relatively high MUFA content (approximately 60% of total fatty acids) and a significant linolenic acid content (approximately 10% of total fatty acids). Sunflower oil was chosen as an comparison oil to canola by virtue of a PUFA content of similar proportions to the MUFA content of canola oil (approximately 70% of total fatty acids) and a minimal linolenic acid content (approximately 0.5% of total fatty acids).

MATERIALS AND METHODS

A. Experimental Design

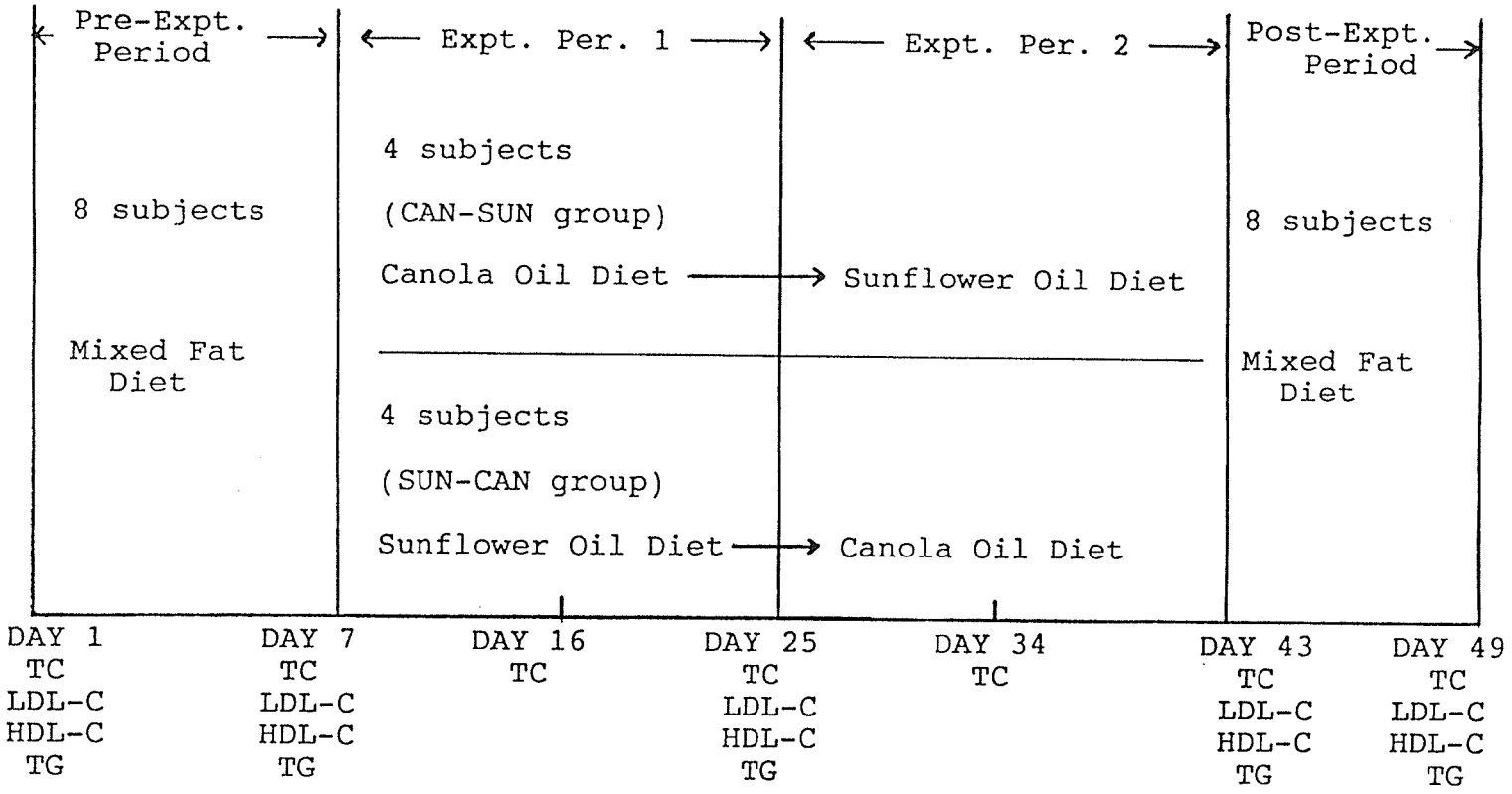
A 48-day metabolic study was divided into 4 periods: a 6-day pre-experimental period ; two 18-day experimental periods; and a 6-day post-experimental period. All subjects received a mixed fat diet during the pre- and post- experimental periods. Four subjects were assigned to the canola oil diet and the other four to the sunflower oil diet during the first experimental period. The subjects on the canola oil diet (CAN-SUN group) were switched to the sunflower oil diet and those on the sunflower oil diet (SUN-CAN group) were switched to the canola oil diet during the second experimental period.

Venous blood samples were obtained from each subject following a 12 hour over-night fast on days 1, 7, 16, 25, 34, 43 and 49. Plasma lipid and lipoprotein analyses were performed on fresh samples. Platelet poor plasma was prepared and stored at -10 C until required for used in phospholipid and CE analyses. The experimental design is shown in Figure 4.

B. Subjects

Eight male subjects were selected from volunteer students who express interest in participating the this study by responding to posted notices. All subjects were evaluated for health status by the University's Health

Figure 4: Experiemental Design



Services; all had plasma cholesterol levels within normal ranges and none had a family history of CHD. Ages ranged from 19 to 32 years and Body Mass Index (kg/m^2) from 20.0 to 28.3. Table 1 summarizes the physical data on the subjects.

The protocol for the study was approved by the Faculty of Human Ecology Ethics Committee. All subjects were required to give written consent for the study. The consent forms used are shown in appendix 1.

Subjects maintained their usual activity patterns and resided in their own homes throughout the study. Meals were served in the metabolic lab in the Human Ecology Building on the University of Manitoba campus. Meals were served at customary times, but accommodated individual schedules as necessary. Emphasis was placed on the fact that no foods, other than those provided were to be eaten. Intakes were regulated to maintain constant body weight. Consumption of alcohol was prohibited. Subjects were instructed against the use of aspirin and were instructed to consult with the project director before taking any medication. Subjects weighed themselves daily, before breakfast.

C. Diets

The nutritionally adequate diets differed only in fat source. A two-day menu cycle was made up of conventional foods (Table 2). All meals and snacks were provided in

TABLE 1

Physical Data

Subject	Ht (cm)	Initial Weight (Kg)	BMI Kg/m^2	Screening Cholesterol mmol/L
1	174.4	61.4	20.0	5.59
2	176.3	79.0	25.5	4.45
3	181.3	83.4	25.4	3.29
4	177.5	77.3	24.5	5.52
5	175.0	86.6	28.3	5.02
6	151.3	54.0	23.6	5.28
7	168.1	70.9	25.1	4.45
8	170.0	67.3	23.3	4.09

(1) Plasma total cholesterol levels 2 weeks prior to the study

TABLE 2

MENU

DAY 1 (1)		DAY 2 (1)	
BREAKFAST		BREAKFAST	
orange juice	(125 ml)	orange juice	(125 ml)
granola (2)	(40g)	granola (2)	(40 g)
bread	(2 slices)	skim milk	(125 ml)
skim milk	(125 ml)		
LUNCH		LUNCH	
chili (2) (4)		sliced chicken (5)	(60 g)
lettuce	(20 g)	rice (2)	(90 g)
oil & vinegar	(5 ml)	bread	(2 slices)
applesauce	(125 ml)	lettuce	(20 g)
skim milk	(250 ml)	tomato	(100 g)
bread	(2 slices)	oil & vinegar	(5 ml)
		fruit cocktail	(125 ml)
		skim milk	(250 ml)
DINNER		DINNER	
chicken casserole (2) (5)		beef pattie(4)	(75 g)
potatoes (2)	(25g-dry)	scalloped potatoes (2)	
carrots	(125 g)	creamed corn	(75 g)
bread	(2 slices)	with oil	(5 g)
peaches	(125 g)	bread	(2 slices)
skim milk	(250 ml)	pears	(125 g)
		skim milk	(250 ml)
SNACK		SNACK	
bread	(2 slices)	bread	(2 slices)
skim milk	(125 ml)	skim milk	(125 ml)
jam	(30 g)	jam	(30 g)
cookies (2)	(4 each)	cookies (2)	(4 each)
spread (3)		spread (3)	

(1) coffee, tea and sugar free soft drinks allowed ad lib. alcohol and other beverages prohibited.
(2) for recipes see appendix 2
(3) The spreads and amount provided daily for the respective diets are described in table 3.
(4) made with ground top round of beef.
(5) breast meat, no skin.

standardized weighed or measured portions for each individual. Mixed dishes, baked items and cereal were prepared according to standardized recipes (Appendix 2). The granola and oatmeal cookies were prepared and weighed into individual portions in advance of the study. The chicken and ground beef for the chili was also cooked and portioned in advance. Entrees, such as the chicken and vegetable casserole, chili and scalloped potatoes were prepared at the time of serving.

The diets provided approximately 3000 Kcals/day. Protein, fat and carbohydrate provided 14.5, 36 and 49.5% of total energy, respectively. Approximately 75% of fat (28% of total energy) came from added fat: a mixture of butter, beef tallow, lard, corn oil and vegetable shortening during the pre- and post- experimental periods; and either canola oil or sunflower oil during the experimental periods. A complete description of the added fat in the three diets is outlined in table 3. The fatty acid composition of the diets is presented in table 4, while the fatty acid composition of the test oils is presented in table 5. Table 6 summarizes the contribution of SFA, MUFA and PUFA to total dietary energy.

Energy intakes were adjusted as appropriate for the maintenance of weight of the subjects throughout the study. Care was taken to keep the proportion of energy from fat and the composition of the dietary fat constant.

D. Storage and Handling of Food Staples

Fats for the mixed fat and experimental diets were purchased in bulk. They were stored in sealed containers at 7 C in a walk-in refrigerator until required for use. Other staples were purchased as single lots from local suppliers and stored under conditions appropriate for each item.

The ground beef for the chili and the chicken were pre-cooked and pre-portioned and stored at -10 C for periods of up to 3 months. Ground beef for the meat patties were portioned raw and also stored at - 10 C. Food items prepared in advance, such as granola and cookies, were also stored in this manner.

Skim milk, bread and fresh vegetables were purchased bi-weekly from a single local source. These items were stored at 7 C in a conventional refrigerator, except the bread which was stored frozen until it was required for use.

TABLE 3

Description of Added Fat

MIXED FAT DIET (g/day)

A mixture of: 22.5 g margarine (18g fat) (1)
18.0 g lard (2)
4.0 g corn oil (3)
27.0 g vegetable shortening (4)
was blended to a uniform consistency and added to baked goods and mixed dishes as described in the recipes in appendix 2.

In addition, 5 g of corn oil (3) was consumed as salad oil and 35.5 g of butter (5) (28.4 g of fat) was consumed as a spread.

CANOLA AND SUNFLOWER OIL DIETS (g/day)

Sixty-seven grams of either canola oil (6) or sunflower oil (7) was added to baked goods and mixed dishes as described in the recipes in appendix 2.

In addition, 5 g of either canola oil (6) or sunflower oil (7) was consumed as a salad oil and 13 g of butter (5) (10.4 g of fat) was blended with 20 g of either canola oil or sunflower oil and consumed as a spread.

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(1) Kroner margarine, (100% beef tallow), Thomas J. Lipton, Toronto, Ont.
(2) Tenderflake Lard, Canada Packers Ltd., Toronto, Ont.
(3) Mazola Corn Oil, Best Foods Division, Canada Starch Co. Ltd., Montreal, PQ.
(4) Crisco Shortening, Procter and Gamble, Toronto, Ont
(5) Manco Butter, Manco Dairies Ltd., Winnipeg, Man.
(6) Canola oil supplied courtesy of CSP Foods, Winnipeg, Man.
(7) Sunflower oil supplied courtesy of CSP Foods, Winnipeg, Man.

TABLE 4

Fatty Acid Composition of Mixed Fat, Canola and Sunflower Oil Diets

Fatty Acid (1)	Fatty Acid Level (% of total fatty acids) Mixed Fat (2)	Canola Oil	Sunflower Oil
14:0	3.6	1.2	1.2
16:0	21.9	8.6	10.6
16:1	2.4	0.9	0.8
18:0	11.2	3.2	5.8
18:1	37.7	53.8	19.5
18:2	18.4	20.8	59.1
18:3	1.2	7.9	0.8
20:0	0.6	0.8	0.5
20:1	0.5	1.3	0.3
22:0	0.2	0.3	0.8
22:1	0.3	-	-

(1) Carbon number:number of double bonds
(2) Added fat included: beef tallow, lard, corn oil, vegetable shortening and butter (table 3).

TABLE 5

Percentage Fatty Acid Composition of the Test Oils

Fatty Acid (1)	Canola Oil	Sunflower Oil
16:0	4.0	6.2
16:1	0.2	–
18:0	1.6	4.5
18:1	59.2	14.5
18:2	21.2	72.7
18:3	10.0	0.6
20:0	0.9	0.5
20:1	1.7	0.3
22:0	0.3	0.7
22.1	0.5	–

(1) Carbon number:number of double bonds

TABLE 6

Contribution of Saturated, Monounsaturated and Polyunsaturated Fatty Acids to Total Energy Intake

	Percent Contribution to Total Energy Intake		
Fatty Acid Group	Mixed Fat Diet	Canola Diet	Sunflower Diet
Saturated	14	5	7
Monounsaturated	15	20	7
Polyunsaturated	7	10	22

E. Lipid and Lipoprotein Analysis

Diets

One-day duplicates of both menus for each of the diets were collected, homogenized in a Waring blender and aliquots taken for lipid analysis. Fat content was determined gravimetrically by the method of Bligh and Dyer (1959). Separate aliquots were extracted by the Bligh and Dyer (1959) method and the fatty acids methylated using BF_3/CH_3OH as described by Metcalfe et al. (1966). The fatty acid methyl esters were analyzed with a Perkin-Elmer (model 3920B) gas chromatograph equipped with dual 2 m x 2mm i.d. glass columns packed with 3% SP-2310 plus 2% SP-2300 on 100/120 Chromosor WAW (Supelco Canada Ltd., Oakville, Canada). Injector, detector and column temperatures were: 250 C, 250 C and 195 C, respectively. The carrier gas was helium. Peak areas were measured with a Hewlett-Packard HP33805 integrator-recorder.

Lipoproteins

Blood samples were collected on days 1, 7, 16, 25, 43 and 49 of the study from subjects fasted for 12 hours. Subjects were comfortably seated while blood was taken from the antecubital vein using vacutainer tubes containing 0.10 mL of 15% solution EDTA(K_3)15 mg. Platelets were removed from the samples by centifuging the tubes at 1400 g. for 15 min. The platelet poor plasma was removed from these samples for analysis. Lipoprotein fractions in the plasma

were separated by ultracentrifugation (Lindgren, 1975) for all samples other than those collected on days 16 and 34. The VLDL fraction and the HDL-fraction were separated by centrifuging 0.6 mL plasma samples at d 1.0063 g/mL and d 1.063 g/mL, respectively, for 16 hours at 104,000 g. Cholesterol was determined enzymatically, in the plasma and in the VLDL and HDL fractions according to the method described by Allain et al. (1986) using a diagnostic kit (Fisher Scientific, Ottawa, Canada). Plasma TG also were determined using a diagnostic kit (Fisher Scientific, Ottawa, Canada) based on the enzymatic method described by Fossati and Lorenzo (1982) and McGowan et al. (1983).

Plasma Phospholipid Separation and Analysis

Lipids were extracted from 1 ml of platelet poor plasma using the method of Folch et al. (1957). Phospholipid species were separated using 2-directional thin-layer chromatography (TLC). Each sample was spotted, under nitrogen, onto a heat activated, pre-coated, silica gel 60 (0.25 mm thick) plate (E. Merck) using a 10 uL Hamilton syringe. Plates were run for approximately 2 hours in the first direction using chloroform/methanol/ammonium hydroxide in a ratio of 65/35/5.5 as a solvent system. The plates were dried under nitrogen for 30 minutes and exposed to HCl fumes for 10 minutes, to hydrolyse the alkenylacyl PE, thus producing lyso-phosphatidylethanolamine (LPE) by cleaving the alkenyl

group from the glycerol moiety. Plates were then dried for an additional 30 minutes before being placed in the second solvent system of chloroform/methanol/formic acid in a ratio of 55/25/5. After a run of approximately 2 hours, the developed plates were dipped in a dichlorofluorescein solution, exposed to ammonia fumes and viewed under UV light. Spots were identified using a standard plate provided by the Dept. of Nutritional Sciences, the University of Guelph. PC, PE and LPE spots were scraped from the plate and placed in methylating tubes. Samples were methylated using H_2SO_4 methanol as described by Holub and Skeaff (1987). The fatty acid methyl esters were analyzed using the Perkin Elmer (model 8500) gas chromatograph equipped with a capillary column, 30m x 0.25mm i.d. coated with DB-225 (J&W Scientific, Folsom, Ca.). Injector, detector and column temperatures were 225 C, 225 C and 205 C, respectively. The carrier gas was helium, at a linear velocity of 25 cm/sec. Fatty acid peaks were identified using a standard mix (cat. #GLC-68B, Nu-Chek Prep. Inc., Elysian, Mn.). In addition 20:5, 22:3 and 22:4 were identified using methylated standards for these fatty acids (Nu-Chek U-100-M, U-82-M and U-83-M, respectively). A chromatogram of the standard mix is located in Appendix 3.

Plasma Cholesterol Ester Separation and Analysis

Lipids were extracted from 0.2ml of platelet poor

plasma using the method of Folch et al. (1957). The neutral lipids were separated using TLC. Samples were spotted under nitrogen onto heat activated, pre-coated, silica gel 20 (0.25mm thick) plates (E. Merck) using a 10uL Hamilton syringe. Plates were placed in a solvent system of hexane/isopropyl ether/glacial acetic acid in a ratio of 60/40/3. Run time was approximately 45 minutes. Developed plates were dipped in a dichlorofluorescein solution, dried for 5 minutes and viewed under UV light. The cholesterol esters were identified as the furthest migrating of the 7 lipid fractions separated; cholesterol oleate (Sigma #C9253) was used to confirm this position. These spots were scraped and placed in methylating tubes. Samples were methylated using sodium methoxide as described by Bannon et al.,(1985). The fatty acid methyl esters were analyzed as described above for the phospholipid fractions.

F. Statistical Analysis

The data for all subjects (n=8) were analyzed using a modified analysis of variance of a two-way classification for a completely randomized block design, using diet type as a co-variate. A sample ANOVA table is located in Appendix 4. For each diet group (n=4), means were compared using the Bonferroni procedure (Appendix 5). Both of these analysis were performed using the SAS computer program (1984, 1986 SAS Institute Inc., Cary, N.C.).

RESULTS

A. Subjects

All subjects successfully followed the study protocol and remained in good health. Although there was no formal evaluation of dietary compliance, other than weight maintenance, subjects remained motivated and committed throughout. The weight of 5 subjects remained essentially constant, while subjects 3, 5 and 7 lost 2.7 Kg, 3.0 Kg and 2.5 Kg, respectively. Although these loses were moderate and extended over a period of 48 days decreases in body weight have been associated with decreases in serum cholesterol (Nestle et al, 1969).

The allocation of subjects to experimental groups resulted in the four individuals with highest plasma TC being assigned to the canola-sunflower oil (CAN-SUN) group and those with the lowest TC levels allocated to the sunflower-canola oil (SUN-CAN) group (Figure 5). However, this situation did not appear to affect the relative responses of the diet groups to the treatment regimens.

B. Effect of Diet on Plasma Lipid and Lipoprotein Levels

The pre-experimental period (mixed fat diet) was employed to standardize intakes among subjects and to allow for energy intake adjustment. TC levels did not change significantly during this period. By contrast, there was a small but significant increase in HDL-cholesterol levels in

the CAN-SUN group. This increase appeared to be transitory as subsequent measurements of HDL-cholesterol were equivalent to initial levels.

There was a significant decrease in plasma TC for both dietary groups during experimental period 1 (Figure 6) (CAN-SUN group 20%, SUN-CAN group 15%). The decrease in TC occurred during the first 9 days of experimental period 1 ; mean TC values on day 16 were 4.06 mM/L and 3.42 mM/L for the CAN-SUN and SUN-CAN groups, respectively (Figure 5). Mean TC levels at the end of experimental period 1, were 3.97 mM/L and 3.39 mM/L for the same treatment groups.

Most of the changes in plasma TC in response to experimental diets was a result of a decrease in LDL-cholesterol (CAN-SUN group 17.1%, SUN-CAN group 13.8%). However, there was also a small but non-significant decrease in HDL-cholesterol for both groups (CAN-SUN group 3.2% and SUN-CAN group 2.8%) (Figure 6).

Switching the experimental fats during experimental period 2 did not result in further changes in TC, LDL, and HDL-cholesterol (Figure 6; Table 7). The results suggest that there was no order effect due to diet on these blood parameters. This is further supported by the similar pattern of change for both diet groups during the study.

Total plasma cholesterol and LDL-cholesterol increased during the post-experimental (mixed fat diet) period. Although the mean values for TC, LDL-and HDL- cholesterol

Figure 5.

Total Plasma Cholesterol Levels of Individual Subjects

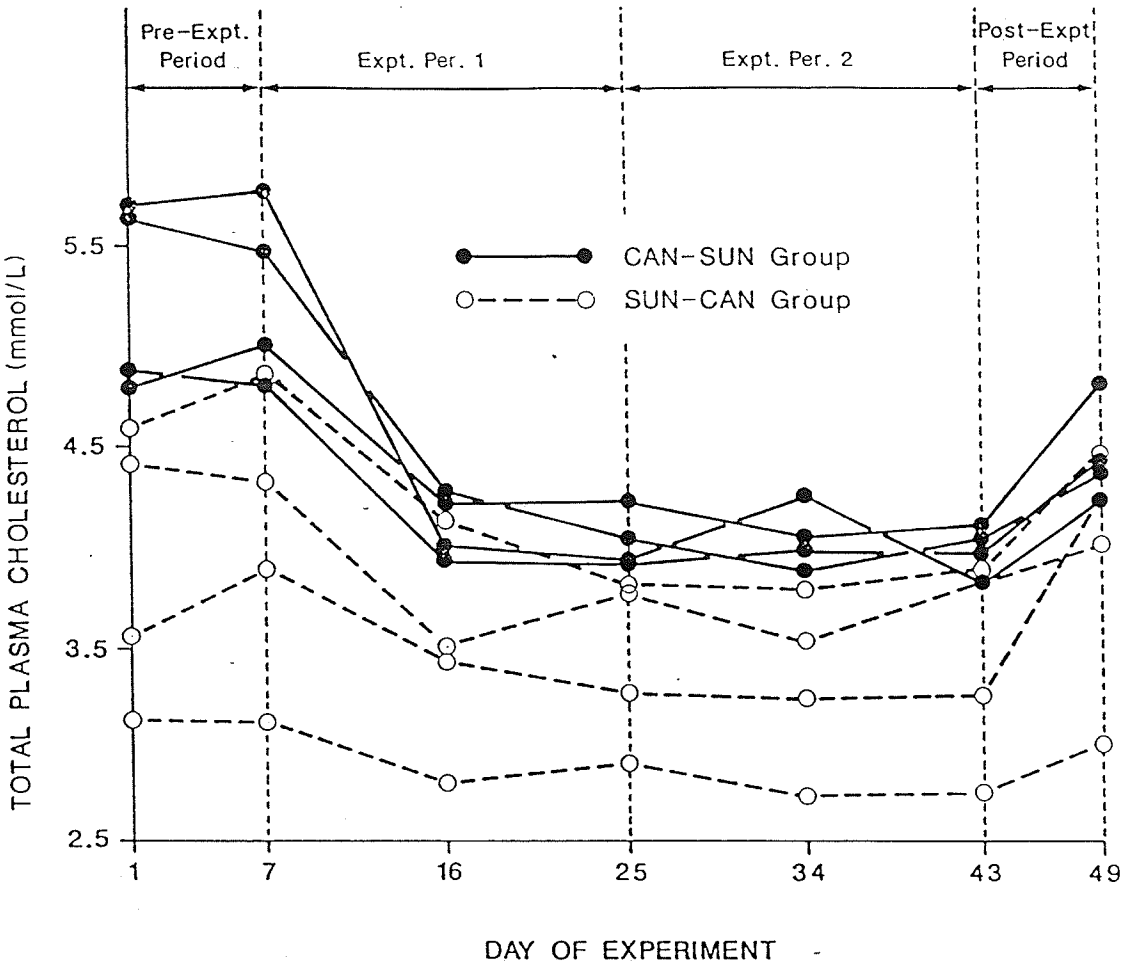
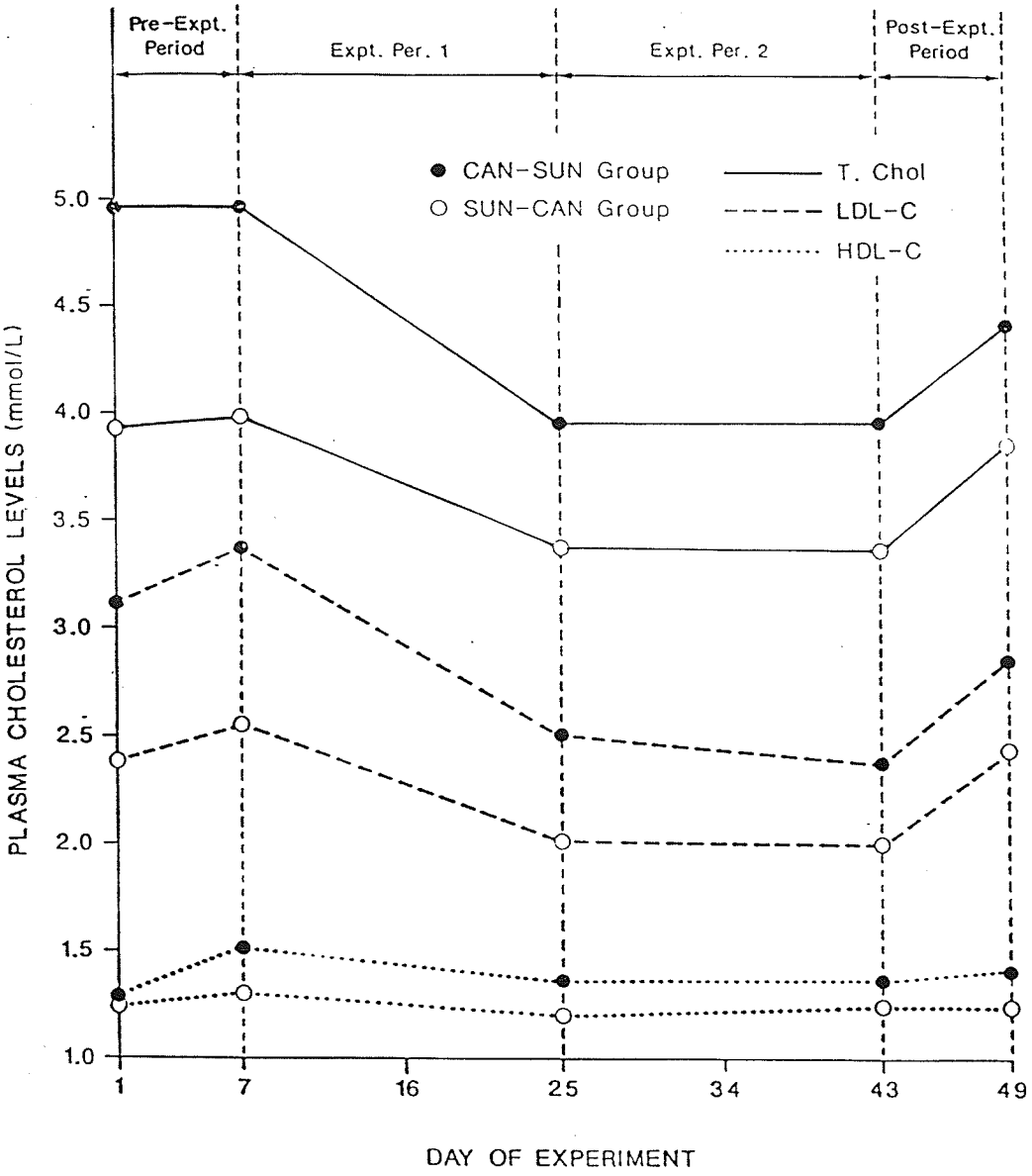


Figure 6

Mean Plasma Cholesterol Levels in Response to Canola and Sunflower oil Diets



T.Chol: Total Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol, HDL-C: High Density Lipoprotein Cholesterol

TABLE 7

Mean Plasma Lipid and Lipoprotein Values

	Mean Level Of Constituent (mmol/ L)*				
	Day 1	Day 7	Day 25	Day 43	Day 49
		Pre-Expt	Expt	Expt	Post-Expt
End of/		Period	Period I	Period II	Period
CAN-SUN Group					
Total Cholesterol	4.95+0.20^{a}†	4.97+0.20^{a}	3.97+0.16^{b}	3.93+0.13^{c}	4.42+0.26^{bc}
LDL-Cholesterol	3.14+0.07^{ab}	3.37+0.41^{a}	2.52+0.32^{c}	2.39+0.31^{c}	2.84+0.39^{bc}
HDL-Cholesterol	1.30+0.31^{a}	1.51+0.46^{b}	1.35+0.41^{a}	1.36+0.37^{a}	1.42+0.41^{a}
VLDL-Cholesterol	0.52+0.31^{a}	0.10+0.12^{a}	0.10+0.07^{a}	0.18+0.12^{a}	0.15+0.12^{a}
Triglycerides	1.33+0.80^{a}	1.03+0.41^{a}	0.82+0.23^{a}	0.82+0.27^{a}	0.89+0.13^{a}
SUN-CAN Group					
Total Cholesterol	3.87+0.69^{a}	3.99+0.72^{a}	3.39+0.42^{b}	3.38+0.53^{b}	3.86+0.63^{ab}
LDL-Cholesterol	2.38+0.77^{a}	2.58+0.79^{a}	2.03+0.40^{a}	2.00+0.50^{a}	2.45+0.64^{a}
HDL-Cholesterol	1.24+0.17^{a}	1.31+0.14^{a}	1.19+0.11^{a}	1.24+0.08^{a}	1.22+0.11^{a}
VLDL-Cholesterol	0.26+0.15^{a}	0.09+0.07^{a}	0.17+0.13^{a}	0.14+0.10^{a}	0.20+0.13^{a}
Triglycerides	1.33+0.80^{a}	1.03+0.41^{a}	0.82+0.23^{a}	0.82+0.27^{a}	0.74+0.32^{a}

* Values are expressed as Mean+SD

† Values in rows with different superscript letters differ at $p<0.05$ comparisions made using the Bonferroni Procedure (Appendix 5)

increased during the post experimental (mixed fat) period, the recovery period apparently was not long enough to allow a return to pre-experimental levels (Table 7).

There were no significant changes in plasma triglycerides during the study (Table 7).

In this study, a diet rich in oleic acid produced an equal hypocholesterolemic effect as a diet rich in linoleic acid. The decrease in TC can be accounted for, primarily by a decrease in the LDL fraction. Neither experimental diet appeared to have an effect on HDL-cholesterol or TG levels in these subjects.

C. Effect of Diet on the Fatty Acid Composition of Plasma Phospholipids and Cholesterol Esters

PC exhibited the most dramatic alterations in fatty acid composition with changes in dietary fat (Table 8). For all eight subjects consumption of the canola oil diet was associated with higher mean levels of oleic acid, linolenic acid and EPA ($p < 0.0001$) and lower levels of stearic acid ($p < 0.004$) and linoleic acid ($p < 0.0001$) compared to levels observed following the sunflower oil diet.

When mean levels of fatty acids within diet groups were compared, the effect of dietary fat source on plasma PC fatty acid patterns also is evident (Table 8). In the CAN-SUN group levels of oleic acid and linolenic acid in

plasma PC were significantly higher following the canola oil diet compared to the levels observed after the pre-experimental period. Similarly, mean levels of oleic acid, linolenic acid and EPA were significantly higher while mean levels of stearic acid and linoleic acid were significantly lower following the canola oil diet compared to the sunflower oil regimen. For the SUN-CAN diet group consumption of the sunflower oil diet was not associated with any compositional changes in PC compared to the pre-experimental period. However, mean levels of oleic acid, linolenic acid and EPA were significantly higher while levels of linoleic acid were significantly lower following the canola oil regimen than those observed following sunflower oil diet.

The PE fraction of the plasma phospholipid appeared to be less sensitive to changes in dietary fat source as shown in Table 9. There also appeared to be more inter-subject variation than for the PC fraction. Nevertheless, for all subjects consumption of the canola oil diet was associated with higher mean levels of oleic acid ($p < 0.0001$) and EPA ($p < 0.066$) and lower levels of stearic acid ($p < 0.01$) and arachidonic acid ($p < 0.045$) in the PE fraction compared to levels observed following the sunflower oil diet. Surprisingly, linolenic acid levels were only slightly higher ($p < 0.40$) following the canola oil diet than those observed following the sunflower oil regimen. There was

appreciable individual subject variability for this fatty acid in the PE fraction. Linolenic acid levels for subjects 1, 4 and 7 were higher after consuming the sunflower oil diet compared to the levels following the canola oil diet (Appendix 8).

The comparisons of the mean fatty acid levels within diet groups over days also suggested that there were relatively small differences in PE fatty acid composition related to dietary fat source (Table 9). In both diet groups higher mean levels of oleic acid were observed following the canola oil diet compared to levels observed following either the mixed fat or the sunflower oil regimens. In the SUN-CAN group, the mean arachidonic acid level in the PE fraction was significantly higher following the sunflower oil diet compared to that observed following the pre-experimental period. Palmitic acid levels were lower following the experimental diets compared to the mixed fat diet.

Changes in the fatty acid composition of the LPE fraction, were very similar to those observed for PE as shown in Table 10. Consumption of the canola oil diet by all subjects was associated with higher mean levels of oleic acid ($p < 0.024$) and EPA ($p < 0.016$) and lower levels of arachidonic acid ($p < 0.042$) compared to levels observed following the sunflower oil regimen. Interestingly, DHA also was lower ($p < 0.016$) following the consumption of the

canola oil diet. In contrast to observations for PE, mean levels of stearic acid were higher ($p < 0.10$) following the canola diet compared to the sunflower oil regimen. However, as in the PE fraction there was no difference in linolenic acid levels due to fat source. Here again there was appreciable inter-subject variability. Linolenic acid levels were higher following the sunflower oil diet than following the canola oil diet for subjects 1, 4 and 7 (Appendix 10). Comparisons of means within each diet group over days were unremarkable, showing no significant differences.

Changes in the fatty acid composition of the plasma CE, as shown in Table 11, echo many of the trends observed for the phospholipid fractions. Consumption of the canola oil diet by all subjects resulted in higher levels of oleic acid ($p < 0.04$), linolenic acid ($p < 0.0007$) and EPA ($p < 0.10$) and a lower level of linoleic acid ($p < .05$) compared to levels observed following the sunflower oil diet. No significant differences occurred in the fatty acid composition of plasma CE within diet groups with the exception of higher levels of linolenic acid following the consumption of the canola oil diet in the CAN-SUN group.

D. SUMMARY

Thus, it would appear that in this study the test fats had equal hypocholesterolemic effects and in both cases this effect was primarily a result of decreases in LDL-

cholesterol. By contrast, the test fats had different effects on the fatty acid composition of plasma phospholipids and CE. Consumption of the canola oil diet by all subjects was consistently associated with higher levels of oleic acid and EPA in the phospholipid species analyzed and in the CE compared to levels associated with the sunflower oil diet. The canola oil diet was also associated with higher levels of linolenic acid in PC and CE, however, this was not observed in either the PE or LPE fractions. By contrast the lower levels of arachidonic acid in PE and LPE following the consumption of the canola oil diet compared to the sunflower oil diet were not observed in the PC or CE fractions. The lower levels of DHA following the consumption of the sunflower oil diet was unique for the LPE fraction.

TABLE 8: Plasma Phosphatidylcholine Fatty Acid Composition at Diet Changes *

	MEAN LEVEL OF FATTY ACID (%)**			
	DAY 7	DAY 25	DAY 43	p***
CAN-SUN GROUP				
16:0	30.51+1.51 a+	28.40+2.25 ab	24.49+1.37 b	.49
18:0	13.21+0.62 ab	12.68+1.07 a	15.10+1.19 b	.004
18:1	13.41+0.26 a	16.81+1.31 b	8.85+0.85 c	.0001
18:2	29.08+3.14 a	28.16+3.20 a	35.82+4.22 b	.0001
18:3	0.20+0.07 a	0.72+0.39 b	0.22+0.19 a	.0001
20:4	7.72+1.05 a	6.75+2.26 a	8.79+3.48 a	.13
20:5	0.36+0.14 ab	0.80+0.40 a	0.11+0.05 b	.0001
22:5	0.64+0.06 a	0.69+0.26 a	0.62+0.19 a	.10
22:6	1.86+0.30 a	1.83+0.50 a	2.35+0.93 a	.41
SUN-CAN GROUP				
16:0	26.98+1.93 a	28.36+5.54 a	26.58+1.41 a	
18:0	12.76+1.14 a	13.36+1.34 a	12.61+1.26 a	
18:1	13.98+1.85 ab	10.84+1.45 a	17.60+1.93 b	
18:2	28.81+3.91 ab	32.93+5.28 a	26.99+4.94 b	
18:3	0.16+0.08 a	0.13+0.02 a	0.57+0.13 b	
20:4	9.70+2.87 a	8.41+3.29 a	8.33+3.09 a	
20:5	0.40+0.18 ab	0.25+0.21 a	0.93+0.60 b	
22:5	0.96+0.21 a	0.68+0.28 a	0.94+0.30 a	
22:6	1.90+0.71 a	1.75+0.86 a	1.56+0.54 a	

* Plasma PC data for individual subjects is located in appendix 6, A sample chromatogram for plasma PC is located in Appendix 7

** Values are expressed as Mean+SD

*** p value resulting from the diet comparision (n=8), (canola vs sunflower oil) Appendix4.

+ Values in rows with different letters differ at $p < 0.05$

TABLE 9: Plasma Phosphatidylethanolaomine Fatty Acid Composition at Diet Changes *

	MEAN LEVEL OF FATTY ACID (%)**			
	DAY 7	DAY 25	DAY 43	p***
CAN-SUN GROUP				
16:0	17.01+1.97 a	14.93+3.00 a	14.26+2.49 a	.78
18:0	21.91+2.74 a	23.45+4.23 a	25.13+2.43 a	.01
18:1	14.11+0.84 a	20.62+1.87 b	13.28+2.32 a	.0001
18:2	22.01+7.76 a	18.07+2.44 a	22.30+3.43 a	.19
18:3	0.29+0.23 a	0.75+0.07 a	0.69+0.60 a	.40
20:4	14.29+2.24 a	11.57+2.76 a	13.43+0.63 a	.045
20:5	1.05+1.29 a	0.59+0.17 a	0.05+0.07 a	.066
22:5	1.60+1.24 a	1.11+0.54 a	2.02+1.60 a	.43
22:6	4.17+3.20 a	3.97+2.48 a	4.15+2.21 a	.15
SUN-CAN GROUP				
16:0	25.84+4.00 a	16.70+2.93 b	17.04+1.11b	
18:0	21.04+3.57 a	25.43+3.07 a	20.75+2.17a	
18:1	14.02+1.89 a	13.08+2.54 a	19.60+3.02b	
18:2	15.82+5.64 a	17.02+3.31 a	16.29+5.88a	
18:3	0.95+0.26 a	0.98+0.27 a	1.17+0.20a	
20:4	11.64+4.08 a	15.59+4.18 b	12.76+3.65ab	
20:5	0.50+0.44 a	0.14+0.09 a	0.77+0.37a	
22:5	1.38+0.36 a	1.38+0.22 a	1.67+0.54a	
22:6	3.52+1.76 a	4.24+1.07 a	3.54+1.05a	

* Plasma PE data for individual subjects is located in Appendix 8, A sample chromatogram for plasma PE is located in Appendix 9

** Values are expressed as Mean+SD

*** p value resulting from the diet comparision (n=8), (canola vs sunflower oil) Appendix4.

+ Values in rows with different letters differ at $p < 0.05$

TABLE 10: Plasma Lyso-Phosphatidylethanolaomine Fatty Acid Composition at Diet Changes *

	MEAN LEVEL OF FATTY ACID (%)**			
	DAY 7	DAY 25	DAY 43	p***
CAN-SUN GROUP				
16:0	11.82+7.15 a	13.64+2.42 a	11.12+6.76 a	.33
18:0	7.27+3.96 a	6.08+1.19 a	5.76+3.68 a	.10
18:1	10.93+4.50 a	8.50+5.20 a	7.31+1.74 a	.024
18:2	24.53+4.15 a	18.46+6.92 a	24.66+8.79 a	.91
18:3	1.10+0.90 a	0.50+0.14 a	0.62+0.57 a	.65
20:4	24.72+10.43a	29.29+6.96 a	29.46+2.92 a	.042
20:5	0.93+0.60 a	2.76+1.48 a	0.60+0.10 a	.016
22:5	3.61+1.64 a	4.01+0.87 a	3.08+0.43 a	.97
22:6	8.26+5.69 a	9.85+2.06 a	9.97+1.56 a	.016
SUN-CAN GROUP				
16:0	15.29+3.08 a	10.18+6.77 a	12.32+3.44 a	
18:0	6.77+1.67 a	5.38+1.51 a	6.06+3.98 a	
18:1	10.02+2.68 a	7.24+4.07 a	9.83+1.32 a	
18:2	18.40+6.22 a	17.59+7.07 a	23.05+13.16a	
18:3	1.16+0.16 a	0.95+0.46 a	1.30+0.98 a	
20:4	30.77+7.15 a	34.19+10.49 a	19.24+10.46a	
20:5	1.33+0.37 a	1.10+0.65 a	1.78+1.39 a	
22:5	4.40+1.34 a	4.62+0.73 a	3.64+1.82 a	
22:6	7.44+2.30 a	11.07+1.88 b	4.95+2.17 a	

* Plasma LPE data for individual subjects is located in appendix 10, A sample chromatogram for LPE is located in Appendix 11

** Values are expressed as Mean+SD

*** p value resulting from the diet comparision (n=8), (canola vs sunflower oil) Appendix4.

+ Values in rows with different letters differ at $p < 0.05$

TABLE 11: Plasma Cholesterol Ester Fatty Acid Composition at diet changes

	MEAN LEVEL OF FATTY ACID (%)**			
	DAY 7	DAY 25	DAY 43	p***
CAN-SUN GROUP				
16:0	12.03+1.05 a	17.75+8.45 a	9.33+2.64 a	.07
18:0	1.89+0.83 a	2.15+1.01 a	2.54+1.77 a	.20
18:1	19.87+3.00 a	17.86+6.54 a	13.40+2.46 a	.04
18:2	56.97+1.08 a	50.02+8.27 a	65.70+3.65 a	.05
18:3	0.65+0.43 a	1.88+0.55 b	0.28+0.25 a	.0007
20:3	0.69+0.17 a	0.78+0.94 a	0.37+0.31 a	.91
20:4	5.35+1.56 a	5.18+1.76 a	5.24+2.21 a	.20
20:5	1.12+1.22 a	2.80+3.36 a	0.51+0.43 a	.10
22:6	0.38+0.43 a	0.71+1.15 a	0.09+0.17 a	.51
SUN-CAN GROUP				
16:0	18.09+3.94 a	17.24+4.03 a	17.44+6.99 a	
18:0	6.13+4.28 a	6.20+2.02 a	2.95+3.58 a	
18:1	21.76+3.67 a	17.88+2.54 a	21.41+2.91 a	
18:2	43.29+7.99 a	48.93+8.64 a	47.52+14.49a	
18:3	0.63+0.61 a	0.47+0.34 a	1.31+0.85 a	
20:3	0.87+1.02 a	0.82+1.63 a	0.32+0.23 a	
20:4	4.68+1.04 a	3.65+1.11 a	4.85+2.13 a	
20:5	1.72+1.21 a	1.57+2.12 a	2.19+2.02 a	
22:6	0.69+0.90 a	0.33+0.66 a	0.15+0.20 a	

* Plasma CE data for individual subjects is located in appendix 12
** Values are expressed as Mean+SD
*** p value resulting from the diet comparision (n=8), (canola vs sunflower oil) Appendix4.
† Values in rows with different letters differ at $p < 0.05$

DISCUSSION

Both test diets induced equivalent reductions in TC and LDL-cholesterol, when compared to the mixed fat diet. These data are consistent with the reports of Mattson and Grundy (1985), who used liquid diets, in which fat provided 40% total energy, to investigate the relative influence of PUFA and MUFA on plasma lipids. In normolipidemic subjects, the diet rich in MUFA resulted in a 12.5% and 17.7% decrease in plasma TC and LDL-cholesterol, respectively, while the PUFA-rich diet produced a 17.1% and 15.6% decrease in these variables. In the present study, the MUFA-rich (canola oil) diet resulted in a 20.0% and 17.1% decrease in TC and LDL-cholesterol, respectively, when substituted for a mixed fat diet, while the PUFA-rich (sunflower oil) diet produced a 15.0%, and 13.8% decrease in TC and LDL-cholesterol, respectively, when it replaced the mixed fat diet.

Keys et al. (1957a) proposed that an increase in PUFA would result in a predictable decrease in plasma cholesterol, while a similar change in MUFA would have no effect. In the studies by Keys et al.(1957a) 6 of the 9 test fats contained relatively constant amounts of MUFA compared to the variation in PUFA and SFA in the same test fats. It is therefore possible that any effect of MUFA on plasma cholesterol was masked by this relatively small variation in MUFA among the fat sources used to develop the

prediction equation.

Although another study by Keys et al. (1965) supported their earlier observations, the diets employed in this study varied in total energy from fat (9.5%, 18.3% and 36.3%). The relatively low fat content of two of the three diets may have masked the effect of oleic acid on serum cholesterol. Sirtori et al. (1986) also reported that oleic acid (olive oil) had no effect on TC in subjects fed diets where fat provided 30% of total energy. By contrast, a diet providing 30% of total energy as corn oil resulted in a significant decrease in TC. However, both regimens resulted in a similar ratio of LDL:HDL-cholesterol. This ratio is considered a reliable index of atherogenic risk (Gordon et al., 1981). The lower energy from fat (30% of total energy) and the fact that subjects were hyperlipidemic may account for the differences between the Sirtori study and the present study and that of Mattson and Grundy (1985). In the present study, all diets provided 36% of total dietary energy as fat and there were appreciable changes in dietary fatty acid composition when the test diets were substituted for a mixed fat diet.

Dietary PUFA have increasingly become the subject of concern among nutritionists and clinicians. Suggestions that high intakes of fats rich in linoleic acid can result in significant decreases in HDL-cholesterol have prompted the current interest in alternative energy sources to

replace dietary SFA. The study by Mattson and Grundy (1985), showed that HDL-cholesterol levels were more frequently reduced following the PUFA diet (mean decrease of 9.8%) than on the MUFA diet (mean decrease of 1.2%). In the present study, there was a small decrease in HDL-cholesterol on both the canola oil (3.2%) and the sunflower oil (2.8%) diets during Experimental period 1. The present study differs from the Mattson and Grundy study (1985) in that the contribution of fat to total energy was lower (36% versus 40%) and conventional foods were used rather than formula liquid diets.

Grundy (1986b) and Mensink and Katan (1987) confirmed the hypocholesterolemic effect of oleic acid. Results from the study by Grundy (1986b), where oleic acid provided 28% of the total energy, were similar to those previously reported for subjects fed formula diets (Mattson and Grundy, 1985). Both of these studies suggested that the substitution of SFA with MUFA had a more favourable effect on plasma lipids and lipoproteins than a reduction in dietary fat to 30% of total energy.

In the present study, analysis of the fatty acid composition of selected phospholipid species and of CE showed important differences depending on dietary fat source. Compositional changes in the PC fraction in particular, reflected the diets consumed. The canola oil

diet contained less palmitic acid, stearic acid and linoleic acid and more oleic acid and linolenic acid than the sunflower oil diet (Table 4). Interestingly, the plasma PC fraction also contained less palmitic acid, stearic acid and linoleic acid and more oleic and linolenic acid following the consumption of the canola oil diet when compared to the sunflower oil diet. Furthermore, the magnitude of these compositional changes relative to the differences in the fatty acid composition of both of the test diets was similar (21%, 27%, 43%, 31% and 27% for palmitic, stearic, oleic, linoleic and linolenic acids, respectively). This suggests that dietary fatty acid composition is indeed reflected in PC fatty acid composition. In addition, this data suggests that, perhaps with the exception of oleic acid, the relative rate of incorporation of these fatty acids into PC is similar.

The magnitude of the change in fatty acid composition in the plasma PE and LPE fractions relative to the changes in dietary fatty acids were considerably less than those observed for the PC fraction, (16%, 4%, 30%, 20% and 2% for palmitic, stearic, oleic, linoleic and linolenic acids, respectively, in PE and 16%, -152%, 24%, 23% and 2% for the same fatty acids in LPE). This observation suggests that PE and LPE have lower rates of incorporation for these fatty acids than has PC. Interestingly, the change in the level of stearic acid in LPE was in the opposite direction

to that of the test diets. In addition, there appeared to be differential rates of incorporation of individual fatty acids into the PE and LPE fractions. Delayed incorporation of linolenic acid into PE compared to PC has been reported in the rat (Weiner and Sprecher, 1984). Similar results have also been reported with humans by Adam et al. (1986) using 4 formula diets containing 4 and 0, 4 and 4, 4 and 8, and 4 and 16% of total energy from linoleic and linolenic acid, respectively. These researchers reported a significant incorporation of linolenic acid into the PC fraction of HDL and LDL which correlated with the level dietary linolenic acid. By contrast these same formula diets did not result in significant changes in the fatty acid composition of the PE fraction in human HDL when consumed over a 2 week period. It is conceivable that had the experimental periods extended beyond 18 days in the present study the degree of response to dietary changes in PE and LPE may have approached that observed in PC.

It is interesting to note that despite the relatively high ratios of linolenic acid to linoleic acid used in the study by Adams and co-workers, the resulting changes in fatty acid composition of phospholipid fractions were modest compared to those observed in the present study. The diets used by Adam et al. (1986) provided much higher percentages of total energy from linolenic acid (0, 4, 8, 12 and 16%) than the present study (0.2 and 2.5 % in the

sunflower and canola oil diets, respectively). By contrast, the percent of total dietary energy from linoleic acid was higher in the present study (20 and 5.5% in the sunflower and canola oil diets, respectively). All diets provided 4% of total dietary energy from linoleic acid in the study by Adam et al.(1986).

The magnitude of the changes in fatty acid composition for the plasma CE, with the exception of palmitic acid, in response to changes in the fatty acid composition of the diet were comparable to, or exceeded corresponding responses in the PC fraction (-168%, 109%, 20%, 23% and 48% for palmitic, stearic, oleic, linoleic and linolenic acids, respectively). However, it would appear that relative rates of incorporation of individual fatty acids into CE vary greatly.

Comparison of the results of the present study with the results of other studies on the effects of dietary linolenic acid on the fatty acid composition of lipid fractions is difficult. Unlike the present study, the majority of researchers assessed compositional changes of either total plasma lipids or total plasma phospholipids as opposed to individual phospholipid species. In addition, the fatty acid composition of the diets used in many of these studies was either unknown or was not reported.

In the present experiment, a dietary source of linolenic acid (canola oil diet) affected levels of this fatty acid

and its metabolites in the plasma phospholipids and in plasma CE. In terms of absolute numbers, the incorporation of linolenic acid and EPA in the phospholipid and CE fractions in response to a dietary source of linolenic acid was modest, ranging from 0.12 to 2.29% of total fatty acids. These levels are consistent with those observed in plasma PC, total phospholipids and CE by other researchers. Sanders and Younger (1981) observed a 1.4% increase, from pre-experimental levels, in the EPA level of plasma PC in omnivor subjects consuming a linseed oil supplement for 2 weeks. Consumption of a 30ml/day supplement of linseed oil over a one week period by a lone subject resulted in an increase of 2.2 and 3.0% of linolenic acid and an increase of 0.7 and 0.5% of EPA in the plasma phospholipid and cholesterol esters, respectively (Dyerburg, 1986). Adam et al (1986) observed a significant increase in linolenic acid in human LDL PC and HDL PC and in EPA in HDL PC of a similar magnitude (0.7, 0.7 and 0.6% of total fatty acids, respectively) when the ratio of dietary linoleic acid to linolenic acid was decreased from 4:0 to 4:4. It has been suggested that the relatively small changes in plasma linolenic acid concentrations in response to elevated dietary linolenic acid are related to its preferential oxidation to CO_2 and H_2O (Adam et al., 1986).

In the present experiment, it was not possible to determine if linolenic acid and its metabolites replace any

specific fatty acids in plasma phospholipids. It has been suggested that linolenic acid successfully competes with linoleic acid for desaturation and elongation resulting in low levels of arachidonic acid. Although there was a significant decrease in PC and PE linoleic acid and PC, PE and LPE arachidonic acid following the consumption of the canola oil diet compared with levels observed following the sunflower oil diet, these results are not unexpected given the variation in the linoleic acid content of the two test diets. Other researchers (Mest et al., 1983, Budowski et al., 1984) have reported a decrease in plasma phospholipid arachidonic acid compared to pre-experimental levels following the consumption of linseed oil supplements. In these studies the fatty acid composition of the subjects' diets were not known. By contrast, Lasserre et al. (1985) observed a decrease of arachidonic acid in serum phospholipids following the consumption of a sunflower oil diet compared to levels observed following a milk fat, peanut oil and LEAR oil diet of known fatty acid composition. Interestingly, the LEAR oil diet resulted in similar arachidonic acid levels to those observed in the standard population (Lasserre et al., 1985).

In the present study there did appear to be evidence of antithrombotic effects following feeding of both the canola and sunflower oil diets. Results reported elsewhere (McDonald et al., in press) demonstrated a significant

increase in bleeding time and in prostacyclin production following the canola oil regimen compared to the mixed fat diet. It was suggested that the relatively high level of linolenic acid in the canola oil diet was related to the increased mean bleeding time observed following the consumption of this diet. Interestingly, consumption of the sunflower oil diet resulted in a significant decrease in thromboxane B_2 production and a greater 6-keto-PGF_1 /thromboxane B_2 ratio compared to the mixed fat diet. The apparent discrepancy between the longer bleeding time associated with the canola oil diet and the lower production of thromboxane B_2 associated with the sunflower oil diet might be explained by the unique behaviour of sunflower oil observed in rats (Hornstra, 1982). In this model sunflower oil was associated with seemingly opposing effects. Sunflower oil was more antithrombotic than saturated fat although it was consistently associated with increased collagen-induced platelet aggregation.

Mest et al. (1983) noted depressed prostaglandin synthesis during linolenic acid ingestion, despite observing no changes in the availability of arachidonic acid. These authors concluded that the decrease in prostaglandin production was the result of inhibition of cyclooxygenase by n-3 fatty acids. Renaud et al. (1986a) observed lower clotting activity of washed human platelets and a decreased induced platelet aggregation response to

thrombin in individuals who had a dietary source of linolenic acid compared to controls.

The present study demonstrated that the hypocholesterolemic effect of a diet rich in oleic acid (canola oil diet) was equal to that of a diet rich in linoleic acid (sunflower oil diet). This hypocholesterolemic effect was primarily due to a decrease in the LDL fraction for both regimens. These results are consistent with the reports of others (Mattson and Grundy , 1985, Mensink and Katan, 1987, Grundy, 1986). The present study also demonstrated that the fatty acid composition of the diet is reflected in the fatty acid composition of plasma phospholipids and CE. Results suggest that phospholipids vary in their relative rates of incorporation of different fatty acids and that PC and CE are plasma lipid components that respond relatively quickly to dietary fat changes. In the present study plasma phospholipid and cholesterol ester EPA was significantly increased by a dietary source of linolenic acid (the canola oil diet) compared to levels observed following the absence of dietary linolenic acid (the sunflower oil diet). The antithrombotic effects of the canola oil diet, however, were similar to those of the sunflower oil diet.

SUMMARY AND CONCLUSIONS

The present study compared the effects of canola oil versus sunflower oil on plasma lipids, lipoproteins and the fatty acid composition of plasma phospholipids and CE in 8 healthy young men. The oils were fed as part of a conventional diet and provided 28% of total energy in a diet which contained 14.5%, 36.0% and 49.5% of total energy as protein, fat and CHO, respectively. Saturated fatty acids provided 14, 5 and 7%, MUFA provided 15, 20 and 7% and PUFA provided 7, 10 and 22% of total dietary energy in the mixed fat, canola oil and sunflower oil diets, respectively. The ratios of linoleic to linolenic acid were 2.6 to 1 and 73.9 to 1 in the canola oil and sunflower oil diets, respectively.

The canola oil and sunflower oil diets produced similar decreases in serum cholesterol (20 and 14%, respectively) and LDL-cholesterol (25 and 21%, respectively). Neither fat source affected plasma HDL-cholesterol or TG levels. However, dietary fat source did effect the fatty acid composition of plasma phospholipids and CE. Oleic acid, linolenic acid and EPA were significantly higher ($p < 0.05$) and linoleic acid was significantly lower in the PC fraction, oleic acid was significantly higher and arachidonic acid was significantly lower in the PE fraction, oleic acid and EPA were significantly higher and aracidonic acid and

decosahexaenoic acid were significantly lower in the LPE fraction and oleic acid , linolenic acid and EPA were significantly higher and linolenic acid was significantly lower in CE on the canola oil diet compared to the sunflower oil diet.

In the present study there was evidence of antithrombotic effects following the feeding of both the canola oil and the sunflower oil diets. The canola oil regimen was associated with a significant increase in bleeding time and in prostacyclin production compared to the mixed fat diet while the sunflower oil diet was associated with a significant decrease in thromboxane B_2 production and a greater 6-keto-PGF_1 /thromboxane B_2 ratio compared to the mixed fat diet.

Thus, it would appear that the canola oil and sunflower oil diets had an equal hypocholesterolemic effect and that the canola oil diet resulted in a higher n-3 fatty acid and lower n-6 fatty acid content in plasma phospholipids and CE compared to the sunflower oil diet. These latter changes are associated with functional changes that suggest that the antithrombotic effects of the canola oil and sunflower oils diets were equivalent.

REFERENCES

Adam, O., Wolfram, G., Zollner, N. 1986. Effect of α -linolenic acid in the human diet on linoleic acid metabolism and prostaglandin biosynthesis. J. Lipid Res. 27:421.

Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W., Fu, P.C. 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. 20:470.

Baggio, G., Pagan, A., Maurizio, M., Martini, S., Opportuno, A., Bonanome, A., Ambrosio, G.B., Ferrari, S., Guarini, P., Piccolo, D., Manzato, E., Corrocher, R., Crepaldi, G. 1988. Olive-oil enriched diet: effect on serum lipoprotein levels and biliary cholesterol saturation. Am. J. Clin. Nutr. 47:960.

Bannon, C.D., Craske, J.D., Hilliker, A.E. 1985. Analysis of fatty acid methyl esters with high accuracy and reliability, IV. Fats with fatty acids containing four or more carbon atoms. J.O.A.C.S. 62:1501.

Betterridge, J. 1987. Nutrition and platelet function in atherogenisis. Proc. Nutr. Soc. 46:345.

Bligh, E.G., Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37:911.

Budowski, P., Crawford, M.A. 1985. α -Linolenic acid as a regulator of the metabolism of arachidonic acid: dietary implications of the ratio, n-6 : n-3 fatty acids. Proc. Nutr. Soc. 44:221.

Budowski, P., Trostler, N., Lupo, M., Vaisman, N., Eldor, A. 1984. Effect of linseed oil ingestion on plasma lipid fatty acid composition and platelet aggregability in healthy volunteers. Nutr. Res. 4:343.

Canadian Consensus Conference on Cholesterol : Final Report. 1988. The Canadian consensus conference on the prevention of heart and vascular disease by altering serum cholesterol and lipoprotein risk factors. C.M.A.J. 139:suppl.

Castelli, W.P., Garrison, R.J., Wilson, P.W.F., Abbott, R.D., Kalousdian, S., Kannel, W.B. 1986. Incidence of coronary heart disease and lipoprotein cholesterol levels. J.A.M.A. 256:2835.

Cook, H.E. 1985. Fatty acid desaturation and chain elongation in eucaryotes. Chapter 6 In: Biochemistry of Lipids and Membranes. Eds., Vance, D.E. and Vance, J.E. Benjamin/Cummings Publishing Co. Inc., Menlo Park.

Croset, M., Lagarde, M. 1986. In vitro incorporation and metabolism of eicosapentaenoic and docosahexaenoic acids in human platelets - effect on aggregation. Thromb. Haemost. 56:57.

Dorfman, K. 1985. Dietary manipulation of prostaglandins with special application to heart disease. J. Appl. Nutr. 37:108.

Dyerberg, J. 1986. Linolenate-derived polyunsaturated fatty acids and prevention of atherosclerosis. Nutr. Rev. 44:125.

Dyerberg, J. 1981. Platelet-vessel wall interaction : influence of diet. Philos. Trans. R. Soc. Lond. B294:373.

Dyerberg, J., Bang, H.O., Aagaard, O., 1980. α -linolenic acid and eicosapentaenoic acid. Lancet. 2:199.

Ferretti, A., Judd, J.T., Marshall, M.W., Flanagan, V.P., Roman, J.M., Matusik, E.J. 1985. Moderate changes in linoleate intake do not influence the systemic production of E prostaglandins. Lipids. 20:268-272.

Ferro-Luzzi, A., Strazzullo, P., Scaccini, C., Siani, A., Sette, S., Mariani, M.A., Mastranzo, P., Dougherty, R.M., Iacono, J.M., Mancini, M. 1984. Changing the Mediterranean diet: effects on blood lipids. Am. J. Clin. Nutr. 40:1027.

Folch, J., Lees, M., Sloane Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497.

Fossati, P., Lorenzo, P. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem. 28:2077.

Gordon, T., Kannel, W.B., Castelli, W.B., Pawber, T.R. 1981. Lipoproteins, cardiovascular disease and death; the Frammingham study. Arch. Intern. Med. 141:1128.

Grundy, S.M. 1986(a). Cholesterol and coronary heart disease :a new era. J.A.M.A. 256:2849.

Grundy, S.M. 1986(b). Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. New. Eng. J. Med. 314;745.

Herold, P.M., Kinsella, J.E. 1986. Fish oil consumption and decreased risk of cardiovascular disease: a comparision of findings from animal and human feeding trials. Am. J .Clin. Nutr. 43:566.

Hirai, A., Terano, T., Saito, H., Tamura, Y., Yoshida, S. 1987. Clinical and epidemiological studies of eicosapentaenoic acid in Japan. Chapter 2 In: Proceedings of the A.O.C.S. Short course on polyunsaturated fatty acids and eicosanoids. ed. Lands, W.E.M. American Oil Chemists Society, Champaign,Ill.

Holub, B.J. 1987. Relationship of fish oil consumption to platelet phospholipid and to the phosphatidylcholine: cholesterol acyltransferase reaction in human plasma. Chapter 10 In: Proceedings of the A.O.C.S. Short course on polyunsaturated fatty acids and eicosanoids. ed. Lands W.E.M. American Oil Chemists Society, Champaign, Ill.

Holub, B.J., Skeaff, C.M. 1987. Nutritional regulation of cellular phosphatidylinositol. In: Methods in Enzymology. Vol. 141. eds. Conn, P.M., Means, A.R. Academic Press Inc., Orlando. pp. 234-244.

Hornstra, G., Haddeman, E., ten Hoor, F. 1979. Fish oils, prostaglandins, and arterial thrombosis. Lancet 2:1080.

Hornstra, G. 1982. Dietary fats, prostanoid and arterial thrombosis. Matinus Nijhoff Pub. The Hague, Netherlands.

Hornstra, G., Rand, M.L. 1986. Effect of dietary n-6 and n-3 polyunsaturated fatty acids on the fluidity of platelet membranes in rat and man. Prog. Lipid Res. 25: 637.

Huang, Y.S., Manku, M.S., Kent, B.A., Nassar, B.A., Harrobin, D.F. 1986. A possible mechanism of action of aspirin and other non-steroidal antiinflammatory drugs : inhibition of essential fatty acid metabolism. Prog. Lipid Res. 25:633.

Hwang, D.H., Carroll, A.E. 1980. Decreased formation of prostaglandins derived from arachidonic acid by dietary linolenate in rats. Am. J. Clin. Nutr. 33:590.

Jacotot, B., Lasserre, M., Mendy, F. 1986. Effects of different diets rich in polyunsaturated fatty acid on plasma phospholipids in the human. Prog. Lipid Res. 25:185.

Keys, A., Anderson, J.T., Grande, F. 1957(a). Prediction of serum-cholesterol responses of man to changes in fats in the diet. Lancet. 2:959.

Keys, A., Anderson, J.T., Grande, F. 1957(b). "Essential" fatty acids, degree of unsaturation and effect of corn (maize) oil on the serum-cholesterol level in man. Lancet. 1:66.

Keys, A., Anderson, J.T., Grande, F. 1965. Serum cholesterol response to changes in the diet: IV. Particular saturated fatty acids in the diet. Metabolism. 14:776.

Keys, A., 1970. Coronary heart disease in seven countries. Circulation. 40:Suppl 1.

Kirkland, S.J., Buchanan, T., Cowan, I., Hooper, H., Shawyer, C.R. 1986. Dihomo-γ-linolenic acid increases prostacyclin production and reduces platelet thromboxane synthesis in the rat. Prog. Lipid Res. 25:331.

Kritchevsky, D., Tepper, S.A., Klurfeld, D.M., Vesselinovitch, D., Wissler, R.W. 1984. Experimental atherosclerosis in rabbits fed cholesterol-free diets. 12. Comparisions of peanut and olive oils. Atherosclerosis 50:253.

Kromhout, D., Bosschieter, E.B., de Lezenne Coulander, C. 1985. The inverse relationship between fish consumption and 20-year mortality from coronary heart disease. N. Eng. J. Med. 312:1205.

Ku, P., Smith, E., Mao, Y. 1987. Recent Canadian mortality trends. Chronic Diseases in Canada 7:46.

Lands, W.E.M. 1986. Renewed questions about polyunsaturated fatty acids. Nutr. Rev. 44:189.

Lasserre, M., Mendy, F., Spielmann, D., Jacotot, B. 1985. Effects of different dietary intake of essential fatty acids on C20:3w6 and C20:4w6 serum levels in human adults. Lipids 20:227.

Leaf, A., Weber, P.C. 1988. Cardiovascular effects of n-3 fatty acids. New Eng. J. Med. 318:549.

Lindgren, F.T. 1975. Preparative ultracentrifugal laboratory procedures and suggestions for lipoprotein analysis. In: Analysis of Lipids an Lipoproteins. ed. Perkins, E.G. A.O.C.S., Champaign, Ill. pp. 204-224.

Longnecker, G.L. 1982. Prostaglandins and the platelet prostanoid compounds. In: Prostaglandins, organ and tissue specific actions. eds. Greenburg, S., Kadowitz, P.J., Burk, T.F. Marcel Dekker, Inc., New York. pp.255-282.

Marshall, L.A., Johnston, P.V. 1982. Modulation of tissue prostaglandin synthesizing capacity by increased ratios of dietary alpha-linolenic acid to linoleic acid. Lipids 12:905.

Mattson, F.H., Grundy, S.M. 1985. Comparison of effects of dietary saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J. of Lipid Res. 26:194.

McDonald, B.E., Gerrard, J.M., Bruce, V.M., Corner, E.J. 1989. Comparision of the effect of canola oil and sunflower oil on plasma lipids and lipoproteins and on in vivo thromboxane A2 and prostacyclin production in healthy young men. Am. J. Clin. Nutr. (in press).

McDonald, B.E. 1983. Studies with high and low erucic acid rapeseed oils in man. Chapter 20 in: High and Low Erucic Acid Rapeseed oil - Production, Usage, Chemistry and Toxicological Evaluation, Eds, Kramer, J.K.G., Sauer, F.S. and Pigden, W.J. Academic Press, New York. p. 535.

McGowan, M.W., Artiss, J.D., Strandberg, D.R., Zak, B. 1983. A peroxidase coupled method for the colorimetric determination of serum triglycerides. Clin. Chem. 29:538.

Mensink, R.P., Katan, M.B. 1987. Effects of monounsaturated fatty acids versus complex carbohydrates on high density lipoproteins in healthy men and women. Lancet 1:122.

Mest, H.-J., Beitz, J., Heinroth, I., Block, H.-U., Forster, W. 1983. The influence of linseed oil diet on fatty acid pattern in phospholipids and thromboxane formation in platelets in man. Klin. Worchenschr 61:187.

Metcalfe, L.D., Schmitz, A.A., Pelka, J.R. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38:514.

Mustard, J.F., Packham, M.A. 1970. Thromboembolism - a manifestation of the response of blood to injury. Circulation 42:1.

Nestel, P.J. 1987. Polyunsaturated fatty acids (n-3, n-6), Am. J. Clin. Nutr. 45:1161.

Nestel, P.J., Connor, W.E., Rearden, M.F., Connor, S., Wong, S., Boston, R. 1984. Suppression by diets rich in fish oil of very low density lipoprotein production in man. J. Clin. Invest. 74:82.

Nestle, P.J., Whyte, M., Goodman, D.S. 1969. Distribution and turnover of cholesterol in humans. J. Clin. Invest. 48:982.

Renaud, S., Norday, A. 1983. "Small is beautiful";∝-Linolenic acid and eicosapentaenoic acid in man. Lancet 2:1169.

Renaud, S., Godsey, F., Dumont, E., Thevenon, C., Ortchanian, E., Martin, J.L. 1986(a). Influence of long-term diet modification on platelet composition in Moselle farmers. Am. J. Clin. Nutr. 43:136.

Renaud, S., Morazain, R., Godsey, F., Dumont, E., Thevenon, C., Martin, J.L., Mendy, F. 1986(b) Nutrients, platelet function and composition in nine groups of French and British farmers Atherosclerosis 60:37.

Renaud, S., Martin, J.L., Thevenon, C. 1987. Long term effects of dietary linoleic and linolenic acids on platelet functions and lipemia in man and woman. Chapter 6 in: Proceedings of the A.O.C.S. Short course on polyunsaturated fatty acids and eicosanoids, ed. Lands, W.E.M. American Oil Chemists Society, Champaign, Ill.

Ross, R. 1986. The pathogenesis of atherosclerosis - an update. New Eng. J. Med. 314:488.

Sanders, T.A.B., Younger, K.M. 1981. The effect of dietary of w-3 polyunsaturated fatty acids on the fatty acid composition of platelets and plasma choline phosphoglycerides. Br. J.Nutr. 45:613.

Sanders, T.A.B., Sulivan, D.R., Rieve, J., Thompson, G.R. 1985. Triglyceride lowering effect of marine polyunsaturates in patients with hypertriglyceridemia. Arterioslerosis 5:459.

Shepherd, J., Packard, C.J., Patsh, J.R., Gotto, A.M.Jr., Taunton, O.D. 1978. Effects of dietary polyunsaturated and saturated fat on the properties of high density lipoproteins and the metabolism of apolipoprotein A-1. J. Clin. Invest. 60:1582.

Sirtori, C.R., Tremoli, E., Gatti, E., Montanari, G., Sirtori, M., Colli, S., Gianfranceschi, G., Maderna, P., Dentone, C.Z., Testolin, G., Galli, C. 1986. Controlled evaluation of fat intake in the Mediterranean diet: comparative activities of olive oil and corn oil on plasma lipids and platelets in high-risk patients. Am. J. Clin. Nutr. 44:635.

Smith, W.L., Borgeat, P. 1985. The eicosanoids: prostaglandins, thromboxanes, leukotrienes and hydroxy-eicosaenoic acids. In: Biochemistry of Lipids and Membranes Eds. Vance D.E. and Vance J.E. Benjamin/Cummings Publishing Co. Inc., Menlo Park pp. 325-360.

Vega, G.L., Groszek, E., Wolf, R., Grundy, S.M. 1982. Influence of polyunsaturated fats on composition of plasma lipoproteins and apolipoproteins. J. Lipid Res. 23:811.

Weaver, B.J., Holub, B.J. 1985. The relative incorporation of arachidonic acid and eicosapentaenoic acid into human platelet phospholipids. Lipids 20:773.

Weiner, B.H., Ockene, I.S., Levine, P.H., Cuenoud, H.F., Fisher, M., Johnson, B.F., Daoud, A.S., Jarmolych, J., Hosmer, D., Johnson, M.H., Natale, A., Vaudreuil, C., Hoogasian, J.J. 1986. Inhibition of atherosclerosis by cod-liver oil in a hyperlidemic swine model. N. Eng. J. Med. 315:841.

Weiner, T.W., Sprecher, H. 1984. Arachidonic acid, 5, 8, 11-eicosatrienoic acid and 5, 8, 11, 14, 17-eicosapentaenoic acid. Dietary manipulation of the levels of these acids in rat liver and platelet phospholipids and their incorporation into human platelet lipids. Biochim. Biophys. Acta. 79:293.

Zollner, N., Adam, O., Wolfram, G. 1979. The influence of linoleic acid intake on the excretion of urinary prostaglandin metabolites. Res. Exp. Med. 175:149.

Zollner, N. 1986. Dietary linolenic acid in man - an over view. Prog. Lipid Res. 25:185.

APPENDICES

APPENDIX 1

Subject Consent Form

HUMAN NUTRITION RESEARCH PROJECT

DEPARTMENT OF FOODS AND NUTRITION
UNIVERSITY OF MANITOBA

CONSENT FORM

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

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

I understand that three 24-hour urine samples will be collected during the study.

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

Dated_____day of_____19____.

Signature_____.

Witness_____.

APPENDIX 2

CHICKEN CASSEROLE Yield: 1 serving

60 g baked chicken breast, skin removed, diced into small pieces
10 g canola or sunflower oil or 10.7 g fat mix (1)
30 g celery, finely chopped
40 g canned mushrooms
40 g chicken broth

Melt fat in individual casserole dishes.
Add chicken broth, meat and vegetables.
Cover and bake at 350F for 30 minutes.

OATMEAL COOKIES Yield: 30 cookies at 25g each

215 g sifted pastry flour
3 g salt
190 g quick rolled oats
150 g canola oil or sunflower oil or 161 g of fat mix (1)
150 g brown sugar
4.5 g baking soda
4 ml vanilla
50 ml boiling water

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

GRANOLA Yield: 60x40g servings

1 kg	rolled oats
125 g	bran
800 g	canola or sunflower oil or 857 g fat mix (1)
500 g	brown sugar

Preheat oven to 350 F.
Combine all ingredients.
Toast in oven, stirring occasionally, for 20 minutes or until golden brown.
Weight into 40g individual portions and package.

(1) composition of fat mix is described in table 3.

SCALLOPED POTATOES Yield: 1 serving

40 g dehydrated sliced potatoes
20 g flour
2 g dehydrated onion
15 g canola oil or sunflower oil or 16 g of fat mix (1)
250 mL boiling water

Preheat oven to 325F.
Measure fat into a foil container 12 cm x 8 cm x 4 cm.
Add flour and stir into a paste.
Add boiling water and dehydrated onion.
Stir to disperse the fat-starch mixture and dissolve large lumps.
Add dehydrated potatoes, stir.
Bake uncovered in oven for 40 minutes.

MASHED POTATOES Yield: 1 serving

25 g instant potato flakes
5 g canola oil or sunflower oil or 5.4 g fat mix (1)
boiling water

Add small amounts of boiling water to potato flakes while Stirring, until mixture reaches the consistency of mashed potatoes.
Stir in fat.

CHILI Yield: 1 serving

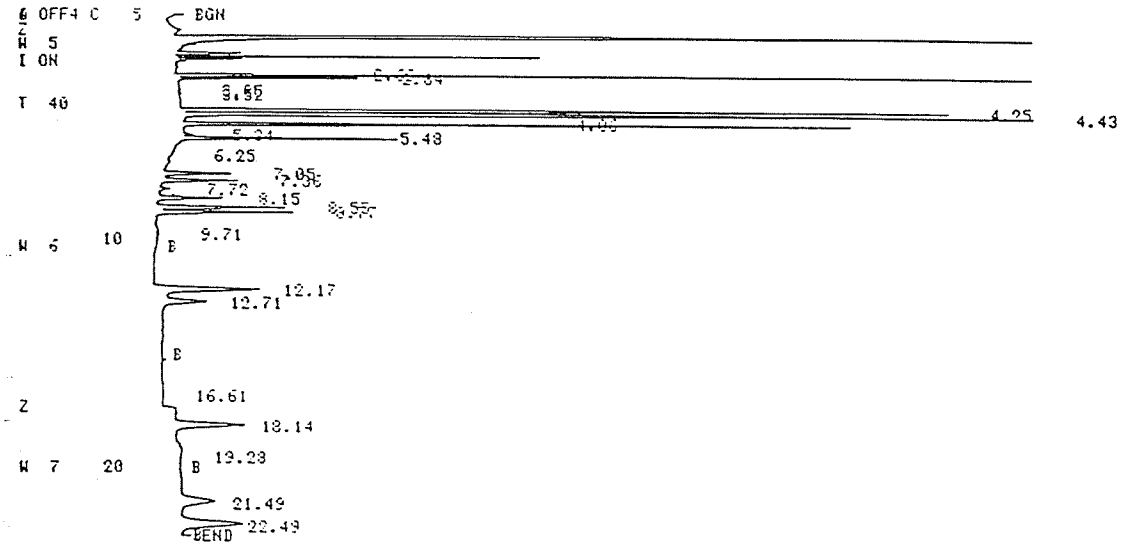
75 g raw ground beef or 59 g cooked ground beef (2)
15 g canola or sunflower oil or 16 g of fat mix (1)
150 g canned tomatoes
75 g kidney beans
30 g onion finely chopped or 3 g dehydrated onion
1.5 ml Worcestershire sauce
2 ml chile powder

Mix all ingredients.
Put in individual casserole dishes.
Cover and bake at 350F for 30 minutes

(1) composition of fat mix is described in table 3.
(2) ground top round of beef.

APPENDIX 3

CHROMATOGRAM OF STANDARD MIX



RETENTION TIME	METHYL FATTY ACID*
N/A	C14:0
N/A	C14:1
2.69	C16:0
2.84	C16:1
4.25	C18:0
4.43	C18:1
4.86	C18:2
5.48	C18:3
7.05	C20:0
7.36	C20:1
8.15	C20:2
8.55	C20:3
8.77	C20:4
12.17	C22:0
12.71	C22:1
18.14	C22:6
21.49	C24:0
22.49	C24:1

* Carbon number: number of double bonds

APPENDIX 4

Sample Analysis of Variance Table - Plasma PC - EPA

SAS

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVEL	VALUES
SUB	8	1 2 3 4 5 6 7 8
DAY	3	7 25 43

NUMBER OF OBSERVATIONS IN DATA SET = 24

SAS

GENERAL LINEAR MODELS PROCEDURE

VARIABLE EPA

	DF	SS	MS	F VALUE	PR>F	R-SQUARE	C.V.
MODEL	10	3.587	0.359	4.88	0.0048	0.78948	55.5532
ERROR	13	0.95674	0.07360		ROOT MSE		EPA MEAN
TOTAL	23	4.54473			0.27128493		2.48833333

	DF	TYPE 1 SS	F VALUE	PR>F	TYPE 3 SS	F VALUE	PR>F
SUBJ	7	1.3047333	2.53	0.0703	1.3047333	2.53	0.0703
DAYS	2	0.1662333	1.13	0.3530	0.1662333	1.13	0.3530
DIET	1	2.1170250	28.77	0.0001	2.1170250	28.77	0.0001

All other ANOVA tables quoted in this thesis are on file in the Department of Foods and Nutrition, University of Manitoba

APPENDIX 5

Sample Bonferroni Means Comparision Procedure - CAN-SUN group - Plasma PC - EPA

ANALYSIS OF VARIANCE PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
SUB	4	1 4 5 6
DAY	3	7 25 43

NUMBER OF OBSERVATIONS IN DATA SET = 12

DEPENDANT VARIABLE: EPA

SOURCE	DF	SS	MS	F VALUE	PR>F	R-SQ	C.V.
MODEL	5	1.726475	0.345295	4.55	0.0463	0.7913	50.89
ERROR	6	0.455550	0.075925		ROOT MSE		EPA MEAN
TOTAL	11	2.182025			0.27554491		0.4525

	DF	SS	F VALUE	PR>F
SUBJ	3	0.444825	1.95	0.2225
DAY	2	1.281650	8.44	0.0180

BONFERRONI (DUNN) T TEST FOR VARIABLE EPA

ALPHA=0.05 CONFIDENCE=0.95 DF=6 MSE=0.075925

CRITICAL VALUE OF T=3.24054 MINIMUM SIGNIFICANT DIF.=0.4054

COMPARISONS SIGN. AT THE 0.05 LEVEL ARE INDICATED BY '***'

DAY COMPARISION	SIMULTANEOUS LOWER CONFIDENCE LIMIT	DIFFERENCE BETWEEN MEANS	SIMULTANEOUS UPPER CONFIDENCE LIMIT	
25-7	-0.1030	0.5375	1.1780	
25-43	0.1420	0.7825	1.4230	***
7-25	-1.1780	-0.5375	0.1030	
7-43	-0.3955	0.2450	0.8855	
43-25	-1.4230	-0.7825	-0.1420	***
43-7	-0.8855	-0.2450	0.3955	

All other Mean Comparisions quoted in this thesis are on file in the Department of Foods and Nutrition, University of Manitoba.

APPENDIX 6

The Fatty Acid Composition of Plasma Phosphotidylcholine (% of total fatty acids) for Individual Subjects

	Fatty Acid					
	16:0			18:0		
Subject DAY/	7	25	43	7	25	43
CAN-SUN GROUP						
1	30.64	31.11	23.58	12.51	13.41	16.52
4	32.30	26.36	23.13	13.82	13.37	15.41
5	30.48	29.37	25.17	13.65	11.12	13.68
6	28.61	26.74	26.06	12.87	12.80	14.79
SUN-CAN GROUP						
2	24.73	21.33	27.54	14.35	15.13	13.26
3	29.16	31.40	27.88	12.58	12.28	10.77
7	27.84	26.80	24.81	11.63	13.66	12.83
8	26.19	33.92	26.08	12.46	12.37	13.57

The Fatty Acid Composition of Plasma Phosphotidylcholine (% of total fatty acids) for Individual Subjects (Cont')

	Fatty Acid					
	18:1			18:2		
Subject DAY/	7	25	43	7	25	43
CAN-SUN GROUP						
1	13.56	18.42	8.30	28.36	24.28	31.80
4	13.17	15.65	9.29	27.66	27.20	34.29
5	13.69	15.83	9.82	26.64	29.35	35.45
6	13.20	17.32	8.00	33.67	31.82	41.72
SUN-CAN GROUP						
2	12.41	12.67	15.07	28.59	28.06	23.54
3	15.13	11.11	19.60	30.46	35.51	31.50
7	12.41	9.20	17.30	32.69	39.15	30.95
8	15.97	10.39	18.41	23.51	29.11	21.96

The Fatty Acid Composition of Plasma Phosphotidylcholine (% of total fatty acids) for Individual Subjects (Cont')

	Fatty Acid					
	18:3			20:3		
Subject DAY /	7	25	43	7	25	43
CAN-SUN GROUP						
1	0.29	0.51	0.14	1.81	1.66	1.84
4	0.20	0.60	0.13	3.12	2.37	2.06
5	0.13	0.48	0.09	3.31	2.20	2.41
6	0.18	1.30	0.50	1.53	1.93	1.42
SUN-CAN GROUP						
2	0.07	0.15	0.70	3.18	3.67	2.56
3	0.16	0.15	0.50	2.49	1.52	1.75
7	0.26	0.11	0.66	2.33	1.09	2.05
8	0.14	0.11	0.42	3.72	1.33	2.36

The Fatty Acid Composition of Plasma Phosphotidylcholine (% of total fatty acids) for Individual Subjects (Cont')

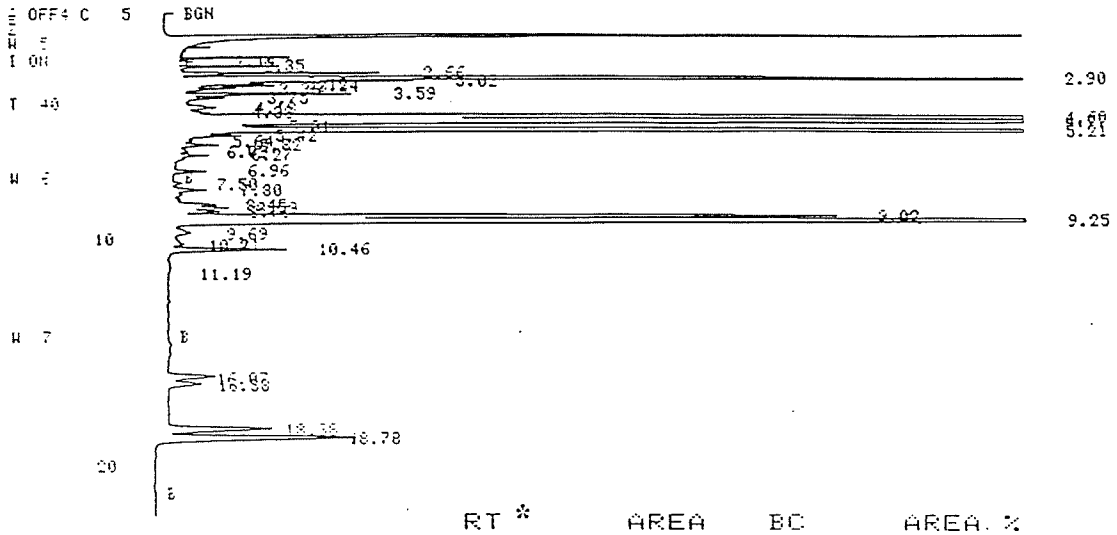
	Fatty Acid								
	20:4			20:5			22:3		
Sub-ject	DAY/ 7	25	43	7	25	43	7	25	43
CAN-SUN GROUP									
1	8.15	6.56	10.37	0.54	1.13	0.09	0.22	0.19	0.72
4	7.19	9.23	11.64	0.36	1.15	0.19	0.25	0.22	0.50
5	7.77	7.41	9.37	0.33	0.58	0.08	0.35	0.17	0.32
6	5.75	3.79	3.76	0.19	0.35	0.08	0.27	0.46	0.43
SUN-CAN GROUP									
2	10.78	12.77	10.36	0.49	0.57	1.73	0.46	0.48	0.30
3	6.64	5.58	4.87	0.22	0.14	0.30	0.37	0.26	0.22
7	8.21	6.19	6.63	0.28	0.16	0.69	0.30	0.30	0.26
8	13.18	9.77	11.44	0.61	0.14	0.98	0.42	0.28	0.50

The Fatty Acid Composition of Plasma Phosphotidylcholine (% of total fatty acids) for Individual Subjects (Cont')

	Fatty Acid								
	22:4			22:5			22:6		
Sub-ject	DAY/7	25	43	7	25	43	7	25	43
CAN-SUN GROUP									
1	0.93	0.00	1.02	0.56	0.63	0.88	1.43	1.19	3.70
4	0.63	0.18	0.00	0.66	1.05	0.63	1.88	2.40	2.17
5	0.33	0.14	0.22	0.69	0.67	0.52	2.00	1.84	1.94
6	0.14	0.00	0.00	0.65	0.42	0.46	2.13	1.89	1.60
SUN-CAN GROUP									
2	0.30	0.33	0.00	0.94	1.07	1.13	2.75	2.84	2.21
3	0.24	0.14	0.10	0.77	0.40	0.53	1.09	0.73	0.90
7	0.17	0.15	0.21	0.86	0.68	0.91	2.13	1.80	1.46
8	0.38	0.18	0.00	1.26	0.58	1.19	1.64	0.98	1.67

APPENDIX 7

SAMPLE CHROMATOGRAM OF PLASMA PC - Subject 8, Day 7



RT *	AREA	BC	AREA. %
2.14	0.0716	T	0.0577
2.35	0.2667	T	0.2149
2.66	0.6035	T	0.4861
2.90	31.1396	T	25.0838
3.02	0.4297	TS	0.3461
3.12	0.1777	TS	0.1432
3.24	0.2543	TS	0.2048
3.36	0.1354	TS	0.1091
3.59	0.6480	T	0.5220
3.75	0.3005	T	0.2421
4.18	0.3782	T	0.3047
4.38	0.2370	T	0.1909
4.60	14.8079	T	11.9281
4.77	18.9786	T	15.2877
5.01	0.0423	TS	0.0341
5.21	27.9587	T	22.5215
5.42	0.1259	TS	0.1014
5.64	0.0304	TS	0.0244
5.82	0.1613	TS	0.1299
6.09	0.0613	TS	0.0494
6.27	0.1758	VS	0.1416
6.96	0.1707		0.1375
7.50	0.0574	T	0.0462
7.80	0.2217	T	0.1786
8.45	0.4797	T	0.3864
8.59	0.3328	T	0.2681
8.76	0.3107	T	0.2503
9.02	4.4176	T	3.5585
9.25	15.6665	T	12.6198
9.69	0.1888	TS	0.1521
10.21	0.1474	TS	0.1188
10.46	0.7233	VS	0.5826
11.19	0.0456		0.0367
16.07	0.4962	T	0.3997
16.38	0.4478	T	0.3607
18.38	1.4988	T	1.2073
18.78	1.9509		1.5715

37 PEAKS > AREA/HT REJECT

* RETENTION TIME

APPENDIX 8

The Fatty Acid Composition of Plasma Phosphotidylethanolamine (% of total fatty acids) for Individual Subjects

	Fatty Acid					
	16:0			18:0		
Subject DAY/	7	25	43	7	25	43
CAN-SUN GROUP						
1	15.14	19.29	13.59	18.03	18.38	23.45
4	19.66	14.13	14.84	22.99	28.72	26.93
5	15.97	13.87	17.29	24.39	23.66	22.66
6	17.27	12.42	11.33	22.22	23.03	27.48
SUN-CAN GROUP						
2	27.31	14.48	18.06	17.09	26.56	18.66
3	20.58	19.45	17.76	20.50	25.55	21.77
7	30.10	19.00	15.62	20.50	25.55	21.77
8	25.36	13.95	16.70	25.76	28.42	23.31

The Fatty Acid Composition of Plasma Phosphotidylethanolamine (% of total fatty acids) for Individual Subjects (Cont')

	Fatty Acid					
	18:1			18:2		
Subject DAY/	7	25	43	7	25	43
CAN-SUN GROUP						
1	14.98	19.66	13.76	33.15	21.44	22.17
4	14.11	23.40	16.35	19.12	17.40	17.51
5	14.40	19.97	11.71	15.24	15.61	25.00
6	12.97	19.43	11.29	20.54	17.82	24.53
SUN-CAN GROUP						
2	12.94	11.52	15.35	13.14	14.06	13.68
3	16.81	16.84	22.26	20.85	20.59	24.42
7	13.53	11.62	21.09	20.14	19.09	16.34
8	12.79	12.32	19.69	9.16	14.36	10.73

The Fatty Acid Composition of Plasma Phosphotidylethanolalamine (% of total fatty acids) for Individual Subjects (Cont')

	Fatty Acid					
	18:3			20:3		
Sub-ject DAY/	7	25	43	7	25	43
CAN-SUN GROUP						
1	tr	0.66	0.77	1.30	0.63	0.78
4	0.55	0.77	1.51	0.86	0.96	0.83
5	0.23	0.74	0.19	1.32	1.02	0.85
6	0.36	0.83	0.30	0.68	1.03	0.85
SUN-CAN GROUP						
2	1.14	0.92	1.02	0.88	0.89	0.79
3	0.69	0.66	0.99	0.86	0.92	0.92
7	1.20	1.31	1.28	0.51	0.57	0.95
8	0.76	1.03	1.39	0.98	0.62	1.26

The Fatty Acid Composition of Plasma Phosphotidylethanolalamine (% of total fatty acids) for Individual Subjects (Cont')

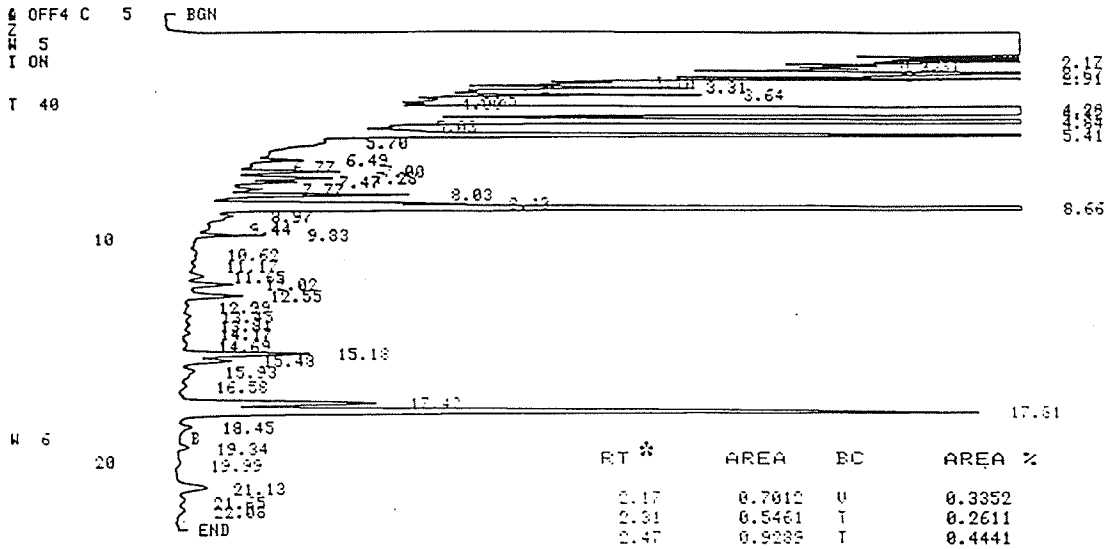
	Fatty Acid								
	20:4			20:5			22:3		
Sub-ject DAY/7	25	43		7	25	43	7	25	43
CAN-SUN GROUP									
1	12.84	9.61	13.91	2.98	0.69	tr	0.00	4.75	4.20
4	11.95	8.93	13.02	0.53	0.34	0.04	2.04	0.76	0.73
5	17.50	14.73	12.77	0.42	0.63	tr	0.67	0.35	2.86
6	14.87	12.99	14.03	0.26	0.71	0.14	0.89	1.02	0.99
SUN-CAN GROUP									
2	9.14	14.97	11.52	1.14	0.12	1.24	1.44	0.96	0.80
3	11.38	11.92	8.84	0.27	0.27	0.36	2.11	0.61	0.36
7	8.56	13.89	13.11	0.17	0.09	0.65	0.82	0.89	0.58
8	17.49	21.56	17.56	0.42	0.09	0.82	0.76	0.94	0.50

The Fatty Acid Composition of Plasma Phosphotidylethanolalamine (% of total fatty acids) for Individual Subjects (Cont')

	Fatty Acid								
	22:4			22:5			22:6		
Sub-ject	DAY/7	25	43	7	25	43	7	25	43
CAN-SUN GROUP									
1	0.00	0.00	0.00	0.00	0.64	4.38	0.00	2.02	1.50
4	0.00	0.40	0.90	3.03	0.66	0.94	3.31	1.63	3.22
5	0.76	0.41	0.00	1.67	1.51	1.20	6.83	6.08	6.38
6	0.50	0.58	0.40	1.71	1.64	1.54	6.52	6.14	5.49
SUN-CAN GROUP									
2	1.65	0.59	1.43	1.75	1.44	1.87	6.12	5.74	4.41
3	1.24	0.39	0.58	1.17	1.54	0.91	2.35	3.57	2.00
7	0.00	0.44	0.39	0.99	1.48	2.18	2.55	4.23	3.79
8	0.60	0.78	0.53	1.61	1.06	1.73	3.07	3.40	3.96

APPENDIX 9

SAMPLE CHROMATOGRAM OF PLASMA PE - Subject 6, Day 43



RT *	AREA	BC	AREA %
2.17	0.7012	V	0.3352
2.31	0.5461	T	0.2611
2.47	0.9285	T	0.4441
2.67	30.5516	T	14.6070
2.79	0.4060	TS	0.1941
2.91	1.9385	TS	0.9273
3.10	0.4141	VS	0.1980
3.31	1.0795	T	0.5161
3.45	0.1018	TS	0.0487
3.64	0.9442	V	0.4514
3.85	0.1765	V	0.0843
4.00	0.1312	T	0.0627
4.29	46.9287	T	22.4370
4.42	23.5059	T	11.2364
4.84	48.4067	T	23.1437
5.00	0.0425	TS	0.0203
5.41	8.6725	T	4.1464
5.70	0.1898	VS	0.0907
6.49	0.2630	T	0.1257
6.77	0.0426	V	0.0204
7.00	0.5799	T	0.2772
7.28	0.6931	T	0.3314
7.47	0.3602	T	0.1722
7.77	0.3287	T	0.1571
8.03	1.2316	V	0.5888
8.43	1.5813	T	0.7560
8.66	22.2309	T	10.6575
8.97	0.1646	TS	0.0787
9.44	0.0721	TS	0.0345
9.83	0.2355	VS	0.1126
10.62	0.1638	T	0.0783
11.17	0.0803	T	0.0384
11.65	0.1820	T	0.0870
12.02	0.4025	T	0.1924
12.55	0.6170	T	0.2950
12.99	0.0538	VS	0.0257
13.45	0.0999	T	0.0477
13.81	0.0960	T	0.0459
14.17	0.0809	T	0.0386
14.69	0.1021	T	0.0488
15.18	1.2379	T	0.5918
15.48	0.5137	T	0.2456
15.93	0.3023	T	0.1445
16.58	0.1083	V	0.0517
17.42	2.2021	T	1.0528
17.81	8.1573	T	3.9001
18.45	0.1419	S	0.0678
19.34	0.1196	V	0.0572
19.99	0.0882	V	0.0421
21.13	0.5491	T	0.2625
21.65	0.1788	T	0.0855
22.08	0.1667	V	0.0797

52 PEAKS > AREA/HT REJECT

* RETENTION TIME

APPENDIX 10

The Fatty Acid Composition of Lyso-Phosphatidylethanolamine (% of total fatty acids) for Individual Subjects

	Fatty Acid					
	16:0			18:0		
Subject DAY/	7	25	43	7	25	43
CAN-SUN GROUP						
1	14.61	10.30	6.57	7.64	5.08	3.19
4	20.41	14.46	19.85	12.10	6.28	10.74
5	8.03	16.02	12.97	6.90	7.68	6.33
6	4.23	13.78	5.07	2.44	5.29	2.76
SUN-CAN GROUP						
2	19.74	19.74	9.16	7.93	4.56	4.89
3	14.94	9.63	16.62	8.46	7.64	11.09
7	13.44	4.04	9.95	5.59	4.48	10.30
8	13.04	7.31	13.54	5.17	4.84	7.94

The Fatty Acid Composition of Lyso-Phosphatidylethanolamine (% of total fatty acids) for Individual Subjects (Cont')

	Fatty Acid					
	18:1			18:2		
Subject DAY/	7	25	43	7	25	43
CAN-SUN GROUP						
1	16.22	10.23	9.38	28.11	13.32	28.35
4	12.10	9.01	7.12	24.47	17.09	14.12
5	9.98	13.52	7.71	26.80	14.83	21.63
6	5.42	11.24	5.10	18.72	28.60	34.55
SUN-CAN GROUP						
2	12.96	8.40	8.48	19.51	12.79	14.09
3	11.30	12.50	11.09	23.77	21.34	33.60
7	8.95	4.09	10.82	20.85	25.60	35.04
8	6.85	3.97	8.92	9.48	10.62	9.47

The Fatty Acid Composition of Lyso-Phosphatidylethanolamine (% of total fatty acids) for Individual Subjects (Cont')

	Fatty Acid					
	18:3			20:3		
Subject DAY/	7	25	43	7	25	43
CAN-SUN GROUP						
1	2.40	0.57	1.23	0.97	0.72	0.18
4	0.66	0.50	0.97	0.38	0.00	0.72
5	0.36	0.63	0.06	0.42	0.84	0.11
6	0.99	0.30	0.20	0.61	0.87	0.95
SUN-CAN GROUP						
2	1.32	1.32	2.71	1.62	0.78	1.78
3	1.26	0.82	0.96	2.27	1.04	1.23
7	0.96	1.31	1.11	1.60	0.74	0.70
8	1.09	0.35	0.43	1.26	0.14	0.13

The Fatty Acid Composition of Lyso-Phosphatidylethanolamine (% of total fatty acids) for Individual Subjects (Cont')

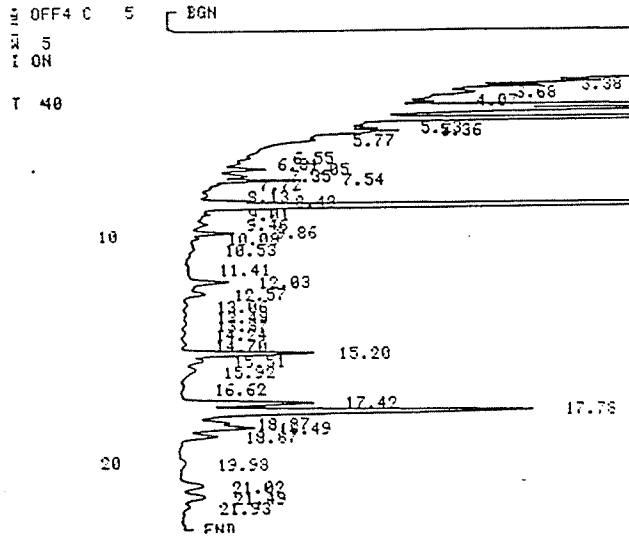
	Fatty Acid								
	20:4			20:5			22:3		
Sub-ject	DAY/7	25	43	7	25	43	7	25	43
CAN-SUN GROUP									
1		16.44	38.57	33.00	0.72	3.98	0.70	1.14	1.42	3.78
4		17.31	29.39	26.20	0.22	4.06	0.59	1.66	2.26	3.61
5		26.26	27.33	28.24	1.15	1.82	0.65	2.74	1.42	1.83
6		38.87	21.86	30.41	1.63	1.17	0.46	2.76	1.42	2.51
SUN-CAN GROUP									
2		32.01	29.45	19.37	0.89	1.23	3.74	5.77	4.03	4.47
3		22.64	25.61	10.19	1.27	1.95	0.64	3.19	1.80	2.02
7		28.63	32.31	13.55	1.80	0.79	0.98	1.66	3.85	0.99
8		39.79	49.37	33.86	1.37	0.44	1.75	3.96	5.04	2.25

The Fatty Acid Composition of Lyso-Phosphatidylethanolamine (% of total fatty acids) for Individual Subjects (Cont')

	Fatty Acid								
	22:4			22:5			22:6		
Sub-jects	DAY/7	25	43	7	25	43	7	25	43
CAN-SUN GROUP									
1	2.66	0.75	2.81	3.33	4.50	2.81	4.26	7.16	8.66
4	0.81	0.00	0.23	2.05	4.94	3.25	4.63	11.99	9.54
5	0.00	0.59	0.56	3.13	3.54	2.65	7.66	9.53	9.45
6	1.03	0.38	0.48	5.92	3.04	3.59	16.49	10.73	12.23
SUN-CAN GROUP									
2	0.00	1.67	11.55	4.16	3.64	5.72	8.94	11.06	6.81
3	1.31	0.67	0.00	2.80	4.74	1.81	4.05	9.26	2.35
7	0.99	1.05	1.79	4.53	5.41	2.44	8.78	13.67	3.95
8	1.36	1.52	0.76	6.06	4.67	4.57	7.99	10.29	6.67

APPENDIX 11

SAMPLE CHROMATOGRAM OF PLASMA LPE - Subject 4, Day 43



RT *	AREA	BC	AREA %
2.22	0.4924	V	0.9143
2.36	0.4955	T	0.9201
2.45	0.2443	T	0.4537
2.52	0.2802	T	0.5203
2.57	0.5404	T	1.0034
2.72	8.9250	T	16.5709
2.84	0.1892	TS	0.3514
2.97	0.9821	TS	1.8235
3.21	0.3261	VS	0.6056
3.38	0.2991	V	0.5553
3.68	0.3078	V	0.5716
4.07	0.1085	T	0.2016
4.28	4.8264	T	8.9611
4.43	3.2054	T	5.9515
4.84	6.3422	T	11.7756
5.23	0.1370	TS	0.2544
5.36	0.4345	TS	0.8068
5.77	0.1057	VS	0.1962
6.55	0.0492	T	0.0914
6.81	0.0465	V	0.0864
7.05	0.2644	T	0.4909
7.35	0.2520	T	0.4679
7.54	0.4998	T	0.9279
7.72	0.0567	VS	0.1052
8.13	0.0724	T	0.1344
8.48	0.3223	T	0.5985
8.70	11.7716	T	21.8562
9.01	0.0725	TS	0.1347
9.46	0.1075	TS	0.1997
9.86	0.2647	TS	0.4915
10.08	0.0588	VS	0.1092
10.53	0.1427	V	0.2649
11.41	0.0791	T	0.1469
12.03	0.5881	T	1.0920
12.57	0.2458	T	0.4564
13.06	0.0632	T	0.1173
13.49	0.0601	T	0.1117
13.87	0.0744	V	0.1382
14.24	0.0600	V	0.1114
14.70	0.0728	T	0.1352
15.20	1.6255	T	3.0181
15.51	0.1047	TS	0.1945
15.92	0.1846	TS	0.3429
16.62	0.0606	TS	0.1125
17.42	1.4642	T	2.7186
17.78	4.2884	T	7.9622
18.27	0.2577	TS	0.4785
18.49	0.8108	T	1.5054
18.87	0.4744	V	0.8808
19.98	0.1784	V	0.3312
21.02	0.3811	T	0.7076
21.49	0.3964	T	0.7361
21.93	0.1636	V	0.3038

53 PEAKS > AREA/HT REJECT

* RETENTION TIME

APPENDIX 12

The Fatty Acid Composition of Plasma Cholesterol Esters (% of total fatty acids) for Individual Subjects

	Fatty Acid					
	16:0			18:0		
Subject DAY/	7	25	43	7	25	43
CAN-SUN GROUP						
1	12.79	25.27	10.34	1.43	3.64	0.92
4	12.88	9.68	12.55	1.75	1.44	2.37
5	11.80	11.21	7.77	1.27	1.71	5.04
6	10.63	24.82	6.67	3.09	1.81	1.82
SUN-CAN GROUP						
2	13.92	18.18	13.69	2.77	3.26	1.03
3	18.87	12.65	16.86	8.52	6.57	1.35
7	16.42	15.88	11.77	10.95	7.84	1.10
8	23.16	22.23	27.44	2.29	7.13	8.31

The Fatty Acid Composition of Plasma Cholesterol Esters (% of total fatty acids) for Individual Subjects (Cont')

		Fatty Acid					
		18:1			18:2		
Subject	DAY/	7	25	43	7	25	43
CAN-SUN GROUP							
1		21.12	17.73	10.36	55.84	37.99	68.27
4		20.03	23.09	14.26	58.23	56.59	63.06
5		22.67	21.94	12.77	56.35	53.85	62.10
6		15.39	8.68	16.19	57.45	51.65	69.37
SUN-CAN GROUP							
2		18.71	14.32	20.04	51.36	46.87	52.72
3		18.79	18.28	25.75	44.87	58.51	51.71
7		26.21	20.32	20.22	32.24	52.22	59.27
8		23.34	18.59	19.61	44.68	38.10	26.37

The Fatty Acid Composition of Plasma Cholesterol Esters (% of total fatty acids) for Individual Subjects (Cont')

	Fatty Acid					
	18:3			20:3		
Sub-ject DAY/	7	25	43	7	25	43
CAN-SUN GROUP						
1	0.38	1.70	0.29	0.64	2.14	0.75
4	0.39	1.41	0.22	0.49	0.53	0.33
5	0.56	1.72	0.61	0.72	0.43	0.40
6	1.28	2.68	0.00	0.91	0.00	0.00
SUN-CAN GROUP						
2	0.86	0.81	1.03	0.46	3.26	0.52
3	0.30	0.59	0.43	0.66	0.00	0.31
7	1.37	0.48	1.33	2.34	0.00	0.43
8	0.00	0.00	2.46	0.00	0.00	0.00

The Fatty Acid Composition of Plasma Cholesterol Esters (% of total fatty acids) for Individual Subjects (Cont')

	Fatty Acid								
	20:4			20:5			22:3		
Sub-ject	DAY/7	25	43	7	25	43	7	25	43
CAN-SUN GROUP									
1	7.22	6.31	7.38	0.57	1.04	0.09	0.00	0.00	0.48
4	4.13	6.01	4.93	0.53	0.81	0.58	0.00	0.00	0.00
5	6.04	5.85	6.34	0.43	1.53	0.29	0.00	0.12	0.66
6	3.99	2.55	2.29	2.94	7.81	1.09	0.00	0.00	0.00
SUN-CAN GROUP									
2	5.85	4.40	7.74	2.13	1.77	2.41	0.00	0.00	0.04
3	3.81	2.25	2.62	1.89	0.00	0.77	0.00	0.00	0.00
7	3.80	3.27	4.49	2.84	0.00	0.60	0.00	0.00	0.00
8	5.27	4.68	4.43	0.00	4.49	4.96	0.00	0.00	0.00

The Fatty Acid Composition of Plasma Cholesterol Esters (% of total fatty acids) for Individual Subjects (Cont')

	Fatty Acid					
	22:4			22:6		
Sub- DAY/ ject	7	25	43	7	25	43
CAN-SUN GROUP						
1	0.00	0.00	0.00	0.00	2.40	0.34
4	0.84	0.00	0.68	0.73	0.44	0.00
5	0.00	0.30	1.99	0.00	0.00	0.00
6	0.63	0.00	0.00	0.77	0.00	0.00
SUN-CAN GROUP						
2	0.93	3.58	0.26	0.86	1.32	0.43
3	0.00	0.98	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	1.90	0.00	0.17
8	0.00	0.00	0.00	0.00	0.00	0.00