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

A THESIS
PRESENTED TO
THE FACULTY OF GRADUATE STUDIES
UNIVERSITY OF MANITOBA

IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE
MASTER OF SCIENCE

DEPARTMENT OF FOODS AND NUTRITION

by

© ELIZABETH J. CORNER

National Library
of Canada

Canadian Theses Service

Bibliothèque nationale du
Canada

Service des thèses
canadiennes

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

BY

ELIZABETH J. CORNER

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

MASTER OF SCIENCE

© 1989

TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd)   PAGE

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.

ACKNOWLEDGEMENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

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.

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

where,

$\triangle$ chol = estimated average change in serum TC (within 2-4 weeks) in mg/100mL.
$\triangle$ S = difference in percent of total energy from dietary saturated fatty acids (SFA).
$\triangle$ P = difference in percent of total energy from dietary polyunsaturated fatty acids (PUFA).
$\triangle$ M = 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.

PHOSPHOLIPID (in cell membrane)

phospholipases

ARACHIDONIC ACID

Cyclooxygenase *

Initiator of
Aggregation PGG_2

Peroxidase

PROSTACYCLIN (PGI_2)←-------PGH_2 --------------→THROMBOXANE
A_2
inhibits platelet   potent platelet
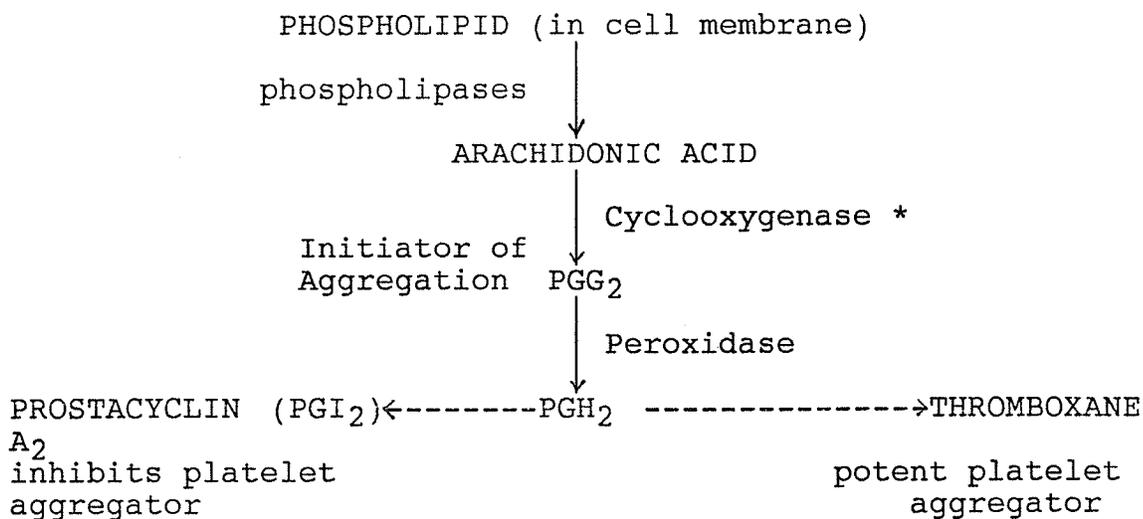aggregator   aggregator

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.

```
16:0--16:1(n-9)      DIET    DIET
  |      |             |       ↓
  |      |             |     18:3(n-3)-*→18:4(n-3)
  |      |             ↓
  |      |        18:2(n-6)-*→18:3(n-6)
  ↓      ↓
18:0--18:1(n-9)---→18:2(n-9)  |      20:4(n-3)-→20:5(n-3)
  |      |             |       ↓
  |      |             |    20:3(n-6)-→20:4(n-6)
  ↓      ↓             ↓
20:0   20:1(n-9)  20:2(n-9)--→20:3(n-9)
  |      |                          |  22:5(n-3)-→22:6(n-3)
  |      |                          |
  ↓      ↓                          |
22:0   22:1(n-9)                    | 22:4(n-6)--→22:5(n-6)
  |      |
  |      |           --→ DESATURATION
  ↓      ↓            ↓   CHAIN ELONGATION
24:0   24:1(n-9)      *   Δ 6 desaturase enzyme
```
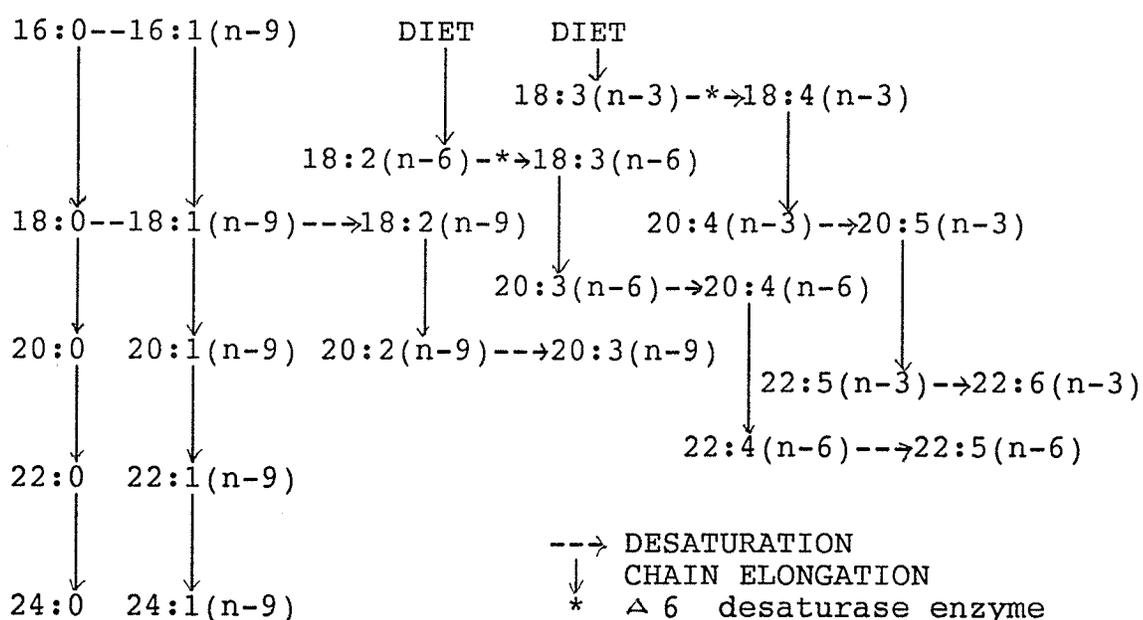
Figure 2. Major pathways of fatty acid biosynthesis by chain elongation and desaturation in animal tissues (Cook, 1985).

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