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

BY

CARRIE RAE MULLIN

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submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
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ABSTRACT

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

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

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LIST OF ABBREVIATIONS

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

ABBREVIATIONS (cont'd).

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

1. LITERATURE REVIEW

1.1 Introduction

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

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

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

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

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

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

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

1.2 Pathology of Atherosclerosis

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

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

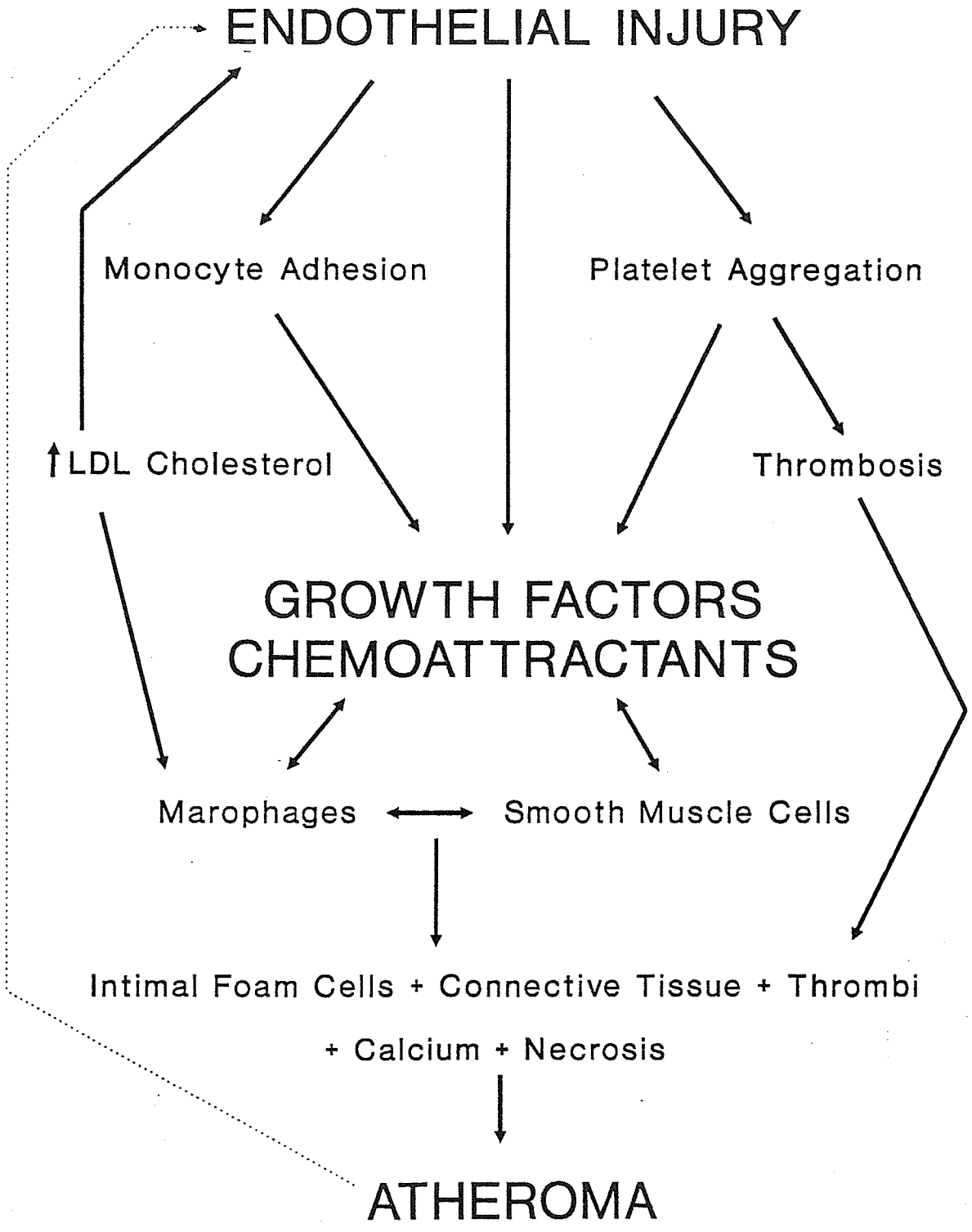

Figure 1. Pathogenesis of Atherosclerosis

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

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

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

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

1.3 Dietary Fatty Acids and Atherosclerosis

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

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

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

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

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

1.3.2.1 SFA

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

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

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

(Grundy, 1987).

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

1.3.2.2 MUFA

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