AN ABSTRACT OF THE THESIS OF

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Title: Effects of Dietary Selenium and Fish Oil (MaxEPA) on Arachidonic Acid Metabolism and Hemostatic Function in the Rat

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The purpose of this study was to investigate whether the beneficial effects which the consumption of fish oil imparts on hemostatic function can be modified by the level of dietary selenium. Male Sprague Dawley rats were fed for eight weeks semipurified diets containing 7% corn oil (by weight) or 5.5% fish oil (MaxEPA) plus 1.5% corn oil with or without selenium supplementation.

The indicators of selenium status (glutathione peroxidase activity and selenium level) were significantly lower in the rats given inadequate selenium, regardless of the type of fat fed. In the animals fed adequate selenium, these same indicators tended to be lower when fish oil was fed. Although feeding of fish oil increased hepatic and aortic malondialdehyde (MDA), selenium supplementation decreased its level in the liver.

Selenium deficiency led to a decrease in the relative weight percent of 22:6 n-3 in aorta and plasma. Increases

in the levels of 20:5 n-3, 22:5 n-3, 22:6 n-3, 20:3 n-6 and a decrease in the level of 20:4 n-6 were observed in plasma total lipids and aortic and hepatic phospholipids when fish oil was fed. The increased level of 20:3 n-6 suggests that delta 5-desaturase activity was decreased by fish oil feeding.

The level of aortic 6-keto-prostaglandin F_1alpha (6-keto-PGF_1alpha) was highest in the rats fed diets that contained corn oil but no selenium supplementation; selenium supplementation, however, eliminated the difference in the level of 6-keto-PGF_1alpha between fish oil and corn oil fed groups. The levels of thromboxane B_2 (TXB_2) and ADP-induced platelet aggregation were decreased significantly by fish oil feeding and tended to be lower with selenium supplementation. Selenium supplementation did not increase bleeding time while fish oil feeding did.

These data indicate that selenium supplementation may decrease fish oil induced lipid peroxidation in liver: this is reflected in the lower hepatic MDA levels in the fish oil fed animals with selenium supplementation and the increased 22:6 n-3 levels in aorta and plasma. Selenium deficiency led to an increase in the level of 6-keto-PGF_1alpha in the rats fed corn oil. Its effects on TXB_2 level and ADP-induced platelet aggregation are marginal. Overall the beneficial effect of selenium supplementation on hemostatic function appears weaker than that of fish oil feeding.

Effects of Dietary Selenium and Fish Oil (MaxEPA) on Arachidonic Acid Metabolism and Hemostatic Function in the Rat

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EFFECTS OF DIETARY SELENIUM AND FISH OIL (MAXEPA) ON ARACHIDONIC ACID METABOLISM AND HEMOSTATIC FUNCTION IN THE RAT

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in the U.S. (U.S. Bureau of the Census, 1988). Great interest has been raised by the claim that the consumption of fish may have a protective value for CVD (Kromhout et al., 1985). Numerous epidemiologic and intervention studies have supported this claim. Among the most significant is the 20-year study done in the Netherlands (Kromhout et al., 1985). In this study an inverse relationship was found between the consumption of fish and death due to heart disease.

The identity of the components of fish that are responsible for its therapeutic value is generally thought to be the long chain, highly unsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in part through their effects on hemostatic function. Numerous studies (Kromhout et al., 1985; Bang & Dyerberg, 1980; Goodnight et al., 1981; Herold & Kinsella, 1986) have shown that these compounds influence platelet aggregability, bleeding time, and production of eicosanoids.

Kromhout et al.(1985), however, speculated that some factor in fish other than these two fatty acids might

contribute to its protective effect. They argue this because the ingestion of relatively small amounts of lean fish, which cannot provide significant quantities of EPA or DHA, appear to decrease cardiovascular risk. Fish, in addition to containing large quantities of these fatty acids, are also rich in selenium (0.4-0.7 ug/g)(Morris & Levander, 1970). Selenium has been suggested as a constituent of fish that may alter hemostatic function (Thorngren & Akesson, 1987) and thus protect against CVD (Salonen et al., 1982). Although there are fewer studies than with EPA and DHA, modification of the level of dietary selenium has been shown to affect thrombosis and prostaglandin synthesis in both human and animal studies (Schiavon et al., 1984; Schoene et al., 1986; Masukawa et al., 1983; Toivanen, 1987). A proaggregatory state can be induced by selenium deficiency and appears to be explained by its role in the synthesis of eicosanoids. To date no studies have attempted to investigate the antiaggregatory effects of the combination of these two nutrients under controlled conditions.

The present study will investigate whether the beneficial effects which consumption of fish oil imparts on hemostatic function can be modified by the levels of dietary selenium. Its specific aims are to determine whether selenium supplemented diets containing fish oils synergystically lower thromboxane synthesis in platelets and increase prostacyclin synthesis in the aorta, compared to

diets containing no selenium and fish oil, corn oil and selenium, or corn oil without selenium; and to determine whether selenium-supplemented diets containing fish oil synergistically lower such thrombotic parameters as platelet aggregation and bleeding time, compared to diets containing no selenium and fish oil, corn oil and selenium, or corn oil without selenium. This study will help to determine if the therapeutic value of fish lies in its EPA content, selenium content, or both.

REVIEW OF LITERATURE

The subject of this review is the current state of knowledge of the modulating roles of polyunsaturated fatty acids (PUFAs) and selenium on arachidonic acid metabolism and hemostatic function.

Nomenclature

In order to facilitate discussion in later sections, the nomenclature for fatty acids and the products of the enzymes to be considered are presented briefly in this section. Dietary fatty acids are classified into families based on the location of the first double bond from the methyl end of the molecule (IUPAC-IUB, 1977). Thus, n-6 fatty acids all have their first double bond between the sixth and seventh carbons from the methyl end. The other fatty acid families are the n-3 and the n-9 families. The designation used to elucidate the molecular structure of fatty acid is as follows: the number before the colon indicates the number of carbon atoms in the fatty acid chain; the number following the colon designates the number of double bonds; the number following the n indicates the family to which it belongs. For example, the notation 18:2 n-6 means that the molecule contains 18 carbons, two double bonds, and that the first double bond is six carbons from

the methyl end. Table 1 lists the fatty acids of relevance to this study by the above designation, the common name and the International Union of Pure and Applied Chemistry (IUPAC) name.

Twenty-carbon polyunsaturated fatty acids containing 3, 4 or 5 double bonds produce eicosanoids via the cyclooxygenase or lipoxygenase pathway (Granstrom, 1987). From the cyclooxygenase pathway are derived prostaglandins and thromboxanes; from lipoxygenase, leukotrienes, lipoxins, and various hydroxy- and hydroperoxy-fatty acids. A summary of the general biosynthetic pathways for the formation of eicosanoids is shown in Figure 1 (Hwang, 1989).

Prostaglandins are oxygenated, polyunsaturated, 20-carbon fatty acids that contain a cyclopentane ring (Figure 2). Each prostaglandin (PG) is designated by a letter (A-J) and a numerical subscript (1,2, or 3). The letter indicates the nature and the position of substituents on the cyclopentane ring and the presence and the position of double bonds within the ring. The carboxyl carbon is C-1. The subscript indicates the number of double bonds in the alkyl side chains. Prostaglandins of the 1-series are derived from 20:3 n-6, those of 2-series from 20:4 n-6 and the 3-series from 20:5 n-3. All prostaglandins posseses an alpha-hydroxy group at C-15 and a C-13, 14-trans double bond. Series 2 and 3 prostaglandins have additional double bonds in the 5, 6 and 17, 18 positions respectively. The structures of the thromboxanes (TXA, TXB) differ slightly

Table 1

Nomenclature of fatty acids

Carbon Number	Common name	IUPAC name[1]
12:0	Lauric acid	Dodecanoic
13:0	Tridecylic acid	Tridecanoic
14:0	Myristic acid	Tetradecanoic
14:1	Myristoleic acid	9-Tetradecenoic
15:0	Pentadecyclic acid	Pentadecanoic
15:1	Pentadecenyl acid	Pentadecenoic
16:0	Palmitic acid	Hexadecanoic
16:1n-7	Palmitoleic acid	9-Hexadecenoic
17:0	Margaric acid	Heptadecanoic
17:1	Heptadecenyl acid	Heptadecenoic
18:0	Stearic acid	Octadecenoic
18:1n-9	Oleic acid	9-Octadecenoic
18:1n-7	Vaccenic acid	11-Octadecenoic
18:2n-6	Linoleic acid	9,12-Octadecadienoic
18:3n-6	Gamma-linolenic acid	6,9,12-Octadecatrienoic
18:3n-3	Linolenic acid	9,12,15-Octadecatrienoic
18:4n-3	Morotic acid	6,9,12,15-Octadecatetraenoic
19:0	-	Nonadecanoic
20:0	Arachidic acid	Eicosanoic
20:1n-9	Gadoleic acid	11-Eicosenoic
20:2n-6	Eicosadienyl acid	11,14-Eicosadienoic
20:3n-6	Dihomo-gamma-linolenic acid	8,11,14-Eicosatrienoic
20:4n-6	Arachidonic acid	5,8,11,14-Eicosatetraenoic
20:5n-3	Timnodonic acid	5,8,11,14,17-Eicosapentaenoic
21:0	-	Heneicosanoic
22:0	Behenic acid	Docosanoic
22:1n-11	Cetoleic acid	11-Docosenoic
22:1n-9	Erucic acid	13-Docosenoic
22:4n-6	Adrenic acid	7,10,13,16-Docosatetraenoic
22:5n-6	-	4,7,10,13,16-Docosapentaenoic
22:5n-3	Clupanodonic acid	7,10,13,16,19-Docosapentaenoic
22:6n-3	Cervonic acid	4,7,10,13,16,19-Docosahexaenoic
24:0	Lignoceric acid	Tetracosanoic
24:1n-9	Nervonic acid	15-Tetracosenoic

[1]IUPAC-International Union of Pure & Applied Chemists.

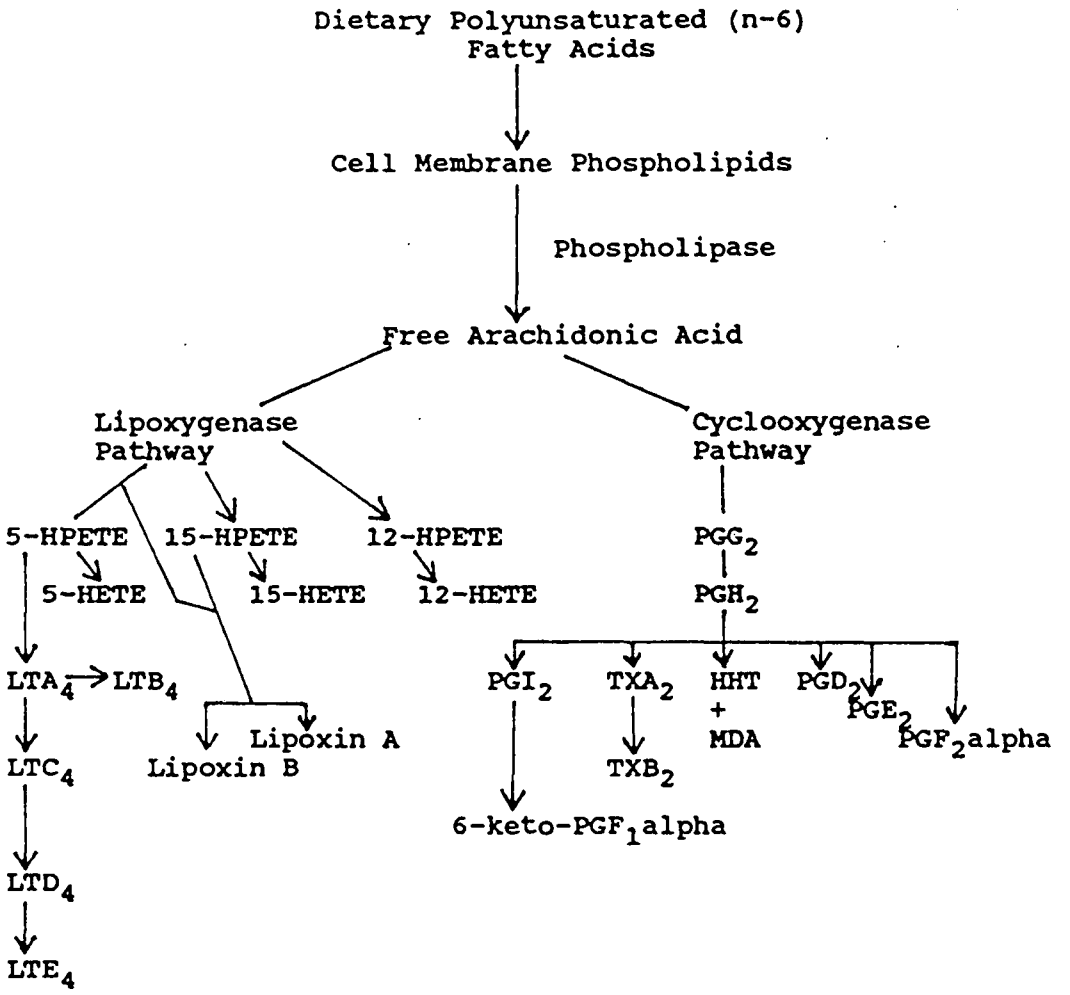

Figure 1. Formation of eicosanoids from arachidonic acid via cyclooxygenase and lipoxygenase pathways. HPETE, hydroxyperoxyeicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; HHT, hydroxyheptadecatrienoic acid, MDA, malondialdehyde.

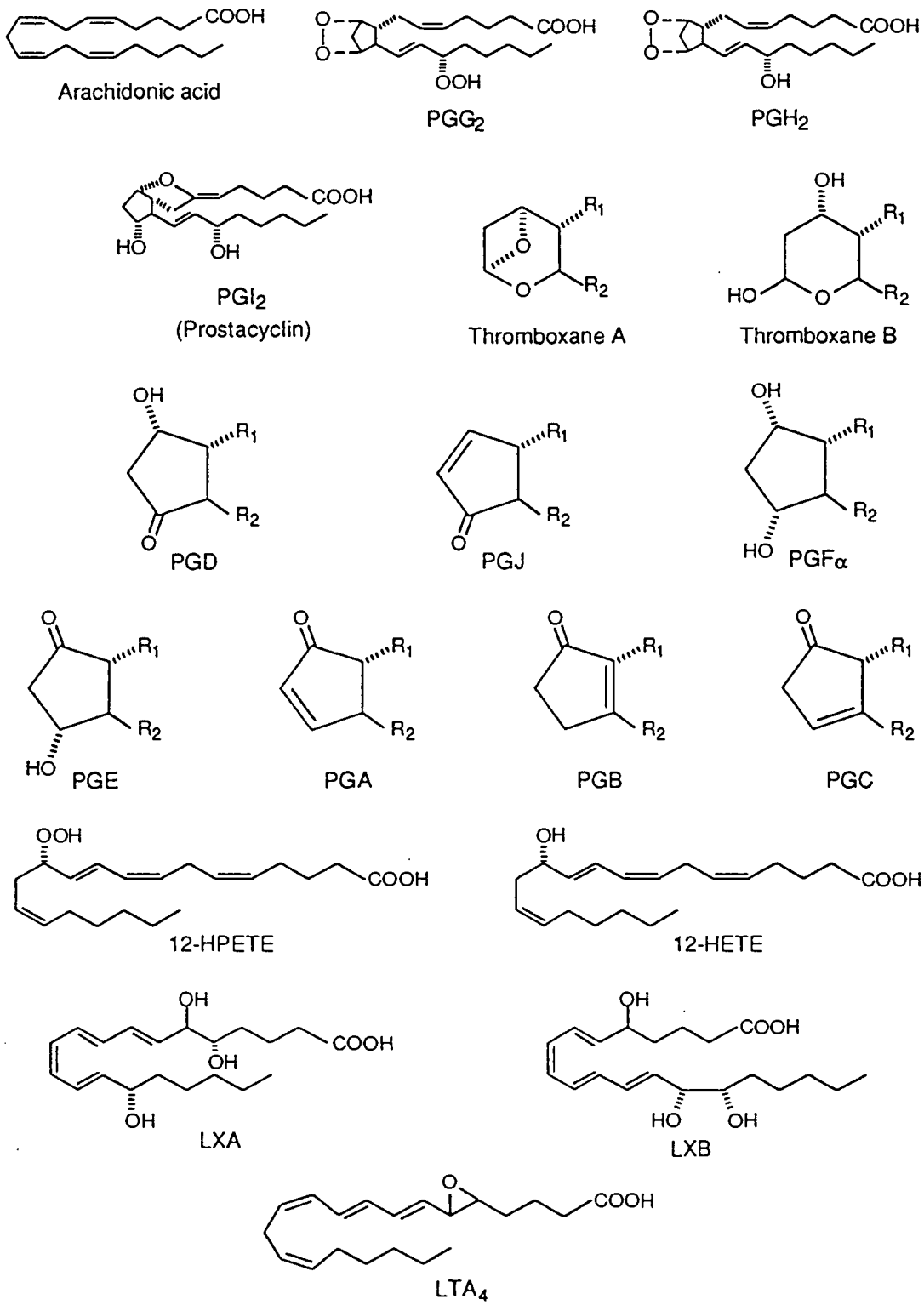

Figure 2. Structures of various eicosanoids. The subscripted α (or β) which is added to prostaglandins of the F-series indicates the spatial position of the hydroxyl group at the C-9 position of the cyclopentane ring.

from those of the prostaglandins: they have a six-membered ring instead of a five-membered one. These structures are also given in Figure 2.

The hydroperoxides are formed by the various lipoxygenses (5-, 12- or 15-lipoxygenases) and are converted to hydroxy fatty acids. Hydroperoxides derived from arachidonic acid are termed hydroperoxyeicosatetraenoic acids (HPETEs) and their hydroxy metabolites, hydroxyeicosatetraenoic acid (HETEs) (Figure 2). Leukotrienes are conjugated trienes produced by the action of 5-lipoxygenase. There are three kinds of leukotrienes: those of the 4-series which are derived from 20:4 n-6; those of the 3-series from 20:3 n-6; and those of the 5-series from 20:5 n-3. The alphabetic designation in the leukortriene name indicates the status of the parent molecule at position 5 and 6 as well as the status of the substituent, if any, on position 6. The structure of the LTA_4 is given in Figure 2. The number of the series is used as a subscript indicating the number of double bonds in the molecule. Lipoxins A and B are members of a recently discovered group of trihydroxy tetraenes derived from arachidonic acid. Their structures are also shown in Figure 2.

Arachidonic Acid Metabolism

Substrate Availability

Conversion of linoleic acid to arachidonic acid.

Linoleic acid (18:2 n-6) is an essential fatty acid and its biologically active form is thought to be arachidonic acid (AA) (20:4 n-6)(Kinsella, 1986a). Small amounts of AA can be obtained directly from the AA content of meat from farm animals; however, most of it is synthesized in the body from linoleic acid (Kinsella, 1986b). The requirement for linoleic acid can be met by having it be 1-2% of the energy intake (i.e 3-7g/day)(Kinsella, 1986a). Current typical intakes in the United States are about six times this amount (Hoskin and Putnam, 1987). The dietary intake of linoleic acid has increased by 55% between 1965 and 1985 (i.e. from 18 to 28 g/capita/day) (Hoskin and Putnam, 1987). Linoleic acid obtained from diets is sequentially desaturated through the removal of four hydrogen atoms and elongated through the addition of two carbon atoms to form AA as shown in Figure 3. The desaturase steps control the synthesis of AA (Kinsella, 1986a).

Arachidonic acid incorporation into and release from phospholipids.

Presumably, extrahepatic tissue such as platelets and endothelial cells obtain polyunsaturated fatty acid by uptake from plasma, the composition of which, in humans, is regulated by liver metabolism (Harris, 1989). Arachidonic acid is esterified along with other fatty acids

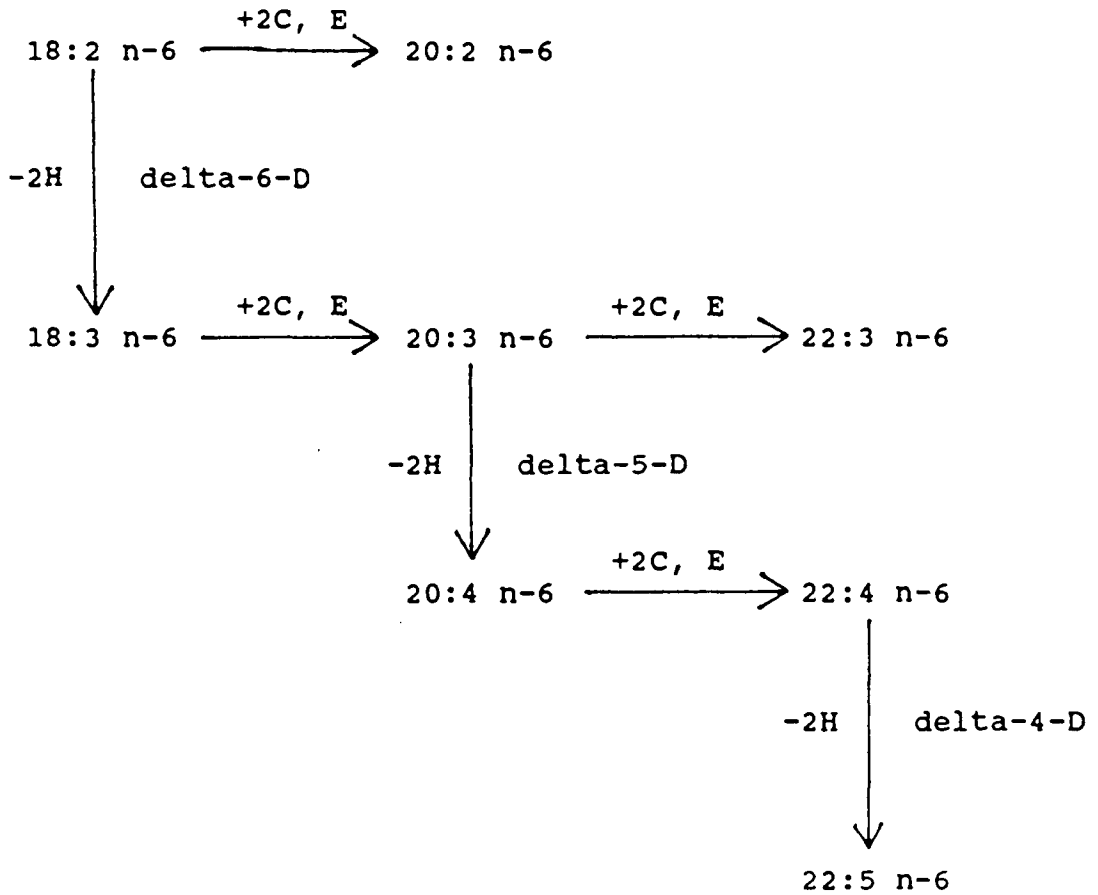

Figure 3. Biosynthetic derivatives of linoleic acid. The +2C means the addition of two carbons. E = elongase; D = desaturase. The number after the delta denotes the position of the carbon from which hydrogen atoms are removed. For example, delta-6-D is a desaturase (D) which removes hydrogen atoms from carbon 6, counted from the carboxyl end of the fatty acid chain.

to become cellular lipids; the highest concentration of arachidonate is generally in phospholipids (especially in sn-2 position), although the greatest total amount may be in triacylglycerols (Lands, 1979).

Activated phospholipases release arachidonic acid from specific membrane phospholipid pools (mostly phosphatidylcholine and phosphatidylinositol). Several agents such as thrombin, collagen, adenosine diphosphate (ADP), epinephrine and divalent cation ionophores activate phospholipases in platelets and endothelial cells (Billah et al., 1979). The release of AA is a rate-limiting step in its metabolism (Panganamala and Cornwell, 1982). Current evidence suggests that AA is released both by the phospholipase A_2 (major pathway) and the phospholipase C-diglyceride lipase pathway (Gerrard et al., 1982; Bills et al., 1976). The phospholipase necesary for release may depend upon the phospholipid to which the fatty acid is esterified. Considerable evidence has accumulated to suggest that phospholipase A_2 is activated for AA release from phosphatidylcholine (Gerrard et al., 1982). The sequential action of phospholipase C and diglyceride lipase may be required to release AA from phosphatidylinositol (Billah et al., 1979; Bell et al., 1979). The metabolites of AA may inhibit its release.

The quantity of AA released reflects the amount present in tissue phospholipids (Kinsella, 1986a). There is some concern that with the high consumption of vegetable oils in

the American diet, tissue phospholipid pools may be saturated with AA (Kinsella, 1986a). Hence, an excess may be released in response to normal physiological stimuli, particularly in diseased and/or stressed states, resulting in overproduction of eicosanoids.

The activity of fatty acids, once they are removed from the phospholipids, may be regulated at several points. The regulation can occur at the interaction with cyclooxygenase or lipoxygenase. It can be by competition at the substrate site with other fatty acids (Culp et al., 1979). For example, AA competes with such fatty acids as eicosapentaenoic acid or docosahexaenoic acid for the substrate binding site of cyclooxygenase. A third point is by the regulation of "peroxide tone". Peroxide tone, as first used by Culp et al. (1979), means the cellular level of peroxide. It can be changed by several factors, including the level of antioxidants.

Enzyme Activities

Cyclooxygenase. The enzyme cycloxygenase (EC 1.14.99.1), located in platelets, vascular cells and many other tissues, catalyzes the insertion of 2 mol of oxygen into and rapid cyclization of arachidonic acid to the endoperoxide, prostaglandin G_2 (PGG_2)(Hamberg et al., 1974). To maintain near-optimal rates of this reaction, tissue oxygen levels must be sufficient (5-10 uM) (Lands, 1979). In addition, lipid hydroperoxides are required. They can,

however, have opposing effects on the enzyme: at low concentrations (10-100 nmol/L), they are activators of fatty acid oxygenation; at higher concentration (1-15 umol/L), they are inhibitory (Warso and Lands, 1983). It is this contradictory behavior of hydroperoxides that led Culp et al. (1979) to coin the phrase peroxide tone. The hydroperoxy endoperoxide formed by cyclooxygenase is reduced to the hydroxy endoperoxide prostaglandin H_2 (PGH_2) by peroxidase activity. These two reactions are catalyzed by prostaglandin endoperoxide synthase, an enzyme which contains both cyclooxygenase and peroxidase activities (Hamberg et al., 1974). One consequence of prostaglandin endoperoxide synthase is that relatively little lipid peroxide, as PGG_2, will be available to maintain the cyclooxygenase in a fully activated state (Cook and Lands, 1976). Any increased removal of PGG_2 (or other lipid peroxides) would tend to reduce the synthesis of prostaglandins.

PGH_2 is converted to a series of different eicosanoids with markedly different physiological effects. It is metabolized to prostaglandin D_2 (PGD_2) by endoperoxide D-isomerase in mast cells and the brain. PGD_2 is known to increase cellular cAMP in platelets. PGH_2 is also metabolized to prostaglandin E_2 (PGE_2) by E-isomerase in most parts of the body including kidney and seminal vesicles. In the kidney it appears to participate in water reabsorption and renin release. Prostaglandin F_2alpha

(PGF_2alpha) is formed by endoperoxidase F alpha-reductase in a variety of tissues from PGH_2 (Lands, 1979). PGE_2 is a vasodilator and bronchodilator, whereas PGF_2alpha is a vasoconstrictor and bronchoconstrictor. Some of the PGH_2 is split into hydroxyheptadecenoic acid (HHT) and malonyldialdehyde (MDA).

PGH_2 can also be transformed into thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) by thromboxane synthase and prostacyclin synthase, respectively. Improved analytical methods have shown that PGI_2 is a major metabolite of arachidonic acid in vascular cells and TXA_2 as a major metabolite in platelets. They share opposing roles in vascular hemostasis. Figure 1 shows a summary of AA metabolites.

Thromboxane synthase. Particular attention has been focused on thromboxane synthase (EC 5.3.99.5) because of the major role that its metabolite, TXA_2, plays in thrombosis. TXA_2 constricts large blood vessels, has variable vasoconstrictor activities in the microcirculation, and is a potent stimulus for platelet aggregation. Thromboxane synthase was first located in human platelets (Moncada et al., 1976a; Needleman et al., 1976) and has been separated from cyclooxygenase (Diczfalusy et al., 1977; Yoshimoto et al., 1977).

Because TXA_2 has an unstable bicyclic oxane-oxetane ring structure, it has a chemical half life of 30 seconds at body pH and temperature (Hamberg et al., 1975). It is

hydrolyzed to TXB_2. TXB_2 can be further metabolized, eventually appearing in the urine primarily as 2,3-dinor-TXB_2. TXB_2 is a relatively stable metabolite from TXA_2 and is commonly measured in plasma as an indicator of TXA_2 levels. Several objections have recently been raised to this practice (Granstrom and Kumlin, 1987). First, since the production of thromboxane is greatly increased in stimulated platelets, a considerable artifactual production may occur during the collection of the blood sample. Second, TXA_2 can undergo metabolic fates other than hydrolysis to TXB_2. Significant amounts of the molecule may appear bound to protein in various ways or it may be dehydrogenated and reduced to 15-keto-13,14-dihydrothromboxane (Granstrom and Kumlin, 1987) and TXB_2 may reflect only a fraction of TXA_2 actually formed. Third, plasma TXB_2 is also cleared rapidly from plasma, although its half life is 10-14 times that of TXA_2 (Nicosia and Patron, 1989). This can lead to an artificially low level of the thromboxanes. This variable seems to be less important than the other two, however, because most plasma TXB_2 levels appear too high (Granstrom, 1986). TXB_2 undergoes further reactions and other metabolites have been suggested as appropriate for measuring TXA_2 production: 11-dehydro-TXB_2 in blood and urine and 2,3-dinor-TXB_2 in urine. Despite these difficulties, TXB_2 production in activated platelets is recognized as a reasonable estimate of their

biosynthetic capacity, because it is the single most prominent product among thromboxane metabolites in blood.

Prostacyclin synthase. Prostacyclin synthase (EC 5.3.99.4) is present in many different tissues including arteries, lung, heart, and uterus (Bunting et al., 1976). The first recognition of the activity of this enzyme was to show that blood vessels convert AA to a substance (PGX) which has very potent antiaggregatory effects on platelets (Moncada et al., 1976a,b; Bunting et al., 1976). This substance was later identified and named prostacyclin (PGI_2)(Moncada, 1976a,b; Bunting et al., 1976). It has opposite effects to TXA_2: inhibition of platelet aggregation and relaxation of vessels. Its structure is shown in Figure 2. Its IUPAC name is 9-deoxy-6,9alpha-epoxy-11,15-dihydroxyprosta-5,13-dienoic acid. PGI_2 has an oxygen bridge between C-6 and C-9. Since the delta-5 double bond is a necessary part of its structure, no PGI_1 can be formed from 20:3 n-6.

Several techniques have been used to measure levels of prostacyclin. In a system generating the compound in vitro, it is often done by immediate assay of the resulting biologic activity, for example by measuring induced relaxation of vessels or by inhibition of platelet aggregation (Moncada and Vane, 1978). Due to the unstable oxygen bridge between C-6 and C-9, PGI_2 is unstable and has a half-life of only 2 min. Consquently assays cannot be based on the measurement of the generation of prostacyclin

in vivo. It is necessary to use a stable metabolite. For a long time, the immediate hydrolysis product, 6-keto-PGF_1alpha has been used for this purpose (Salmon et al., 1978). Plasma 6-keto-PGF_1alpha was measured as an indicator of PGI_2 synthesis in the body and prostacyclin was believed to be a circulating hormone. But the measurement of plasma 6-keto-PGF_1alpha is now thought to be an unreliable indicator of PGI_2 synthesis due to following reasons. Because prostacyclin can be metabolized by many tissues (such as vessel walls and the kidney) into 2,3-dinor-6-keto-PGF_1alpha, 2,3-dinor-6,15-diketo-13,14-dihydro-20-carboxyl-PGF_1alpha and 2,3-dinor-6,15-diketo-13,14-dihydro-PGF_1alpha, the concept of prostacyclin as a circulating hormone was abandoned. The major metabolite in plasma is not 6-keto-PGF_1alpha. Urinary 6-keto-PGF_1alpha was also used as an indicator of PGI_2 synthesis in the body. However, renal 6-keto-PGF_1alpha is only a minor metabolite and reflects mainly the renal PGI_2 synthesis (Granstrom and Kumlin, 1987). As a better choice for a major blood and urinary metabolite, 2,3-dinor 6,15-diketo-13,14-dihydro-20-carboxyl-PGF_1alpha is suggested. The measurement of 6-keto-PGF_1alpha in the incubated tissues such as lung or aorta has not yet been criticized. Though it would not show the in vivo synthesis of PGI_2, it would be a reasonable indicator of the ability of tissues to synthesize PGI_2 because the part which produces prostacyclin is directly used. This measurement is termed ex vivo production.

Lipoxygenase. Lipoxygenases constitute a family of non-heme iron containing dioxygenases that are widely distributed in nature. Lipoxygenase can convert arachidonic acid, which contains the cis, cis-1,4-pentadiene structure, to a variety of compounds which contain hydroperoxy groups at positions 5, 8, 9, 11, 12, or 15 (Hamberg and Samuelsson, 1967). The products formed depend on the cell type. Because the biologic function of many of the lipoxygenase products are not well known, the finding of the 12-lipoxygenase in platelets; the 5-lipoxygenase in polymorphonuclear leukocytes (PMNLs), neutrophils, monocytes, and basophils; and the 15-lipoxygenase in PMNLs, macrophages, eosinophils, and lymphocytes has led to much research. The 5-, 12-, 15-hydroperoxyeicosatetraenoic acid (HPETE) can be reduced almost immediately to the corresponding hydroxyeicosatetraenoic acids (HETEs) by such enzymes as glutathione peroxidase (GSHPx).

Other conversions of the hydroperoxy acids are known. The 5-HPETE can be dehydrated to form epoxides (leukotriene A_4). LTA_4 is converted to leukotriene B_4 (LTB_4) by hydrolase. LTA_4 can be acted on by glutathione-S-transferase and gamma-glutamyl transpeptidase to form the peptolipids leukotriene C_4, D_4 and E_4. The lipoxins are the latest identified substances, formed by the lipoxygenase pathway. Two such compounds are known to date: lipoxin A (LXA), 5,6,15-trihydroxy-7,9,11,13-eicosatetraenoic acid,

and lipoxin B (LXB), 5,14,15-trihydroxy-6,8,10,12-eicosatetraenoic acid (see Figure 2).

The metabolic knowledge in this area is still limited and few assay methods for these compounds have been developed. HPETE may play a role as an endogenous modulator of PGI_2 synthesis (Turk, 1980). Although the lipoxygenase route determines the fate of about one-third of the arachidonate liberated during platelet activation, the role of its products is less well established than those of thromboxane and prostacyclin from the cyclooxygenase pathway. When the cyclooxygenase pathway and lipoxygenase pathway are compared, their relative rates are different: TXA_2 production via the cyclooxygenase pathway peaks rapidly (<min) following platelet stimulation (Hwang, 1982) while increases in 12-HETE and/or 12-HPETE continue for over 1 hour. This was interpreted to indicate substantial potential for the products from the lipoxygenase pathway having effects for a long duration. For instance, they, because they are present for longer periods of time, might modify PGI_2 synthase. Lipoxygenase in blood vessels produces 12-HPETE (Greenwald et al., 1979; Herman et al., 1979) as well as 15-HPETE (Powell and Funk, 1987; Funk and Powell, 1985). These substances, by being lipid peroxides, may play a role as endogenous regulators of PGI_2 synthesis (Turk, 1980). In contrast to the above, Aharony et al. (1982) found that 12-HPETE, and 12-HETE to a lesser extent, down regulated PGH_2/TXA_2-induced aggregation. Croset and

Lagarde (1983) have found that 12-HETE, 15-HETE, and less so, 5-HETE inhibited PGH_2-induced aggregation. LTC_4 and LTD_4 may increase thromboxane production (Marx, 1982). LTC_4 may influence certain bronchial functions. LTB_4 is a potent inducer of chemotaxis and the aggregation of leukocytes. Thus, the leukotrienes are believed to be the important mediators in inflammation and allergy (Marx, 1982). Among the effects of LXA and LXB are stimulation of superoxide anion generation and degranulation of neutrophils (Serhan et al., 1984).

Balance between the Production of Thromboxane and Prostacyclin

The discovery of TXA_2 and PGI_2 has increased the understanding of AA metabolism in vivo and of the homeostatic regulation of normal interactions between platelets and vessel walls. According to the theory proposed by Moncada and Vane (1979), the balanced synthesis and action of the proaggregatory, vasoconstrictor TXA_2 by platelets and antiaggregatory, vasodilator PGI_2 by vascular tissue is important in maintaining the fluidity of circulating blood.

Platelets (or megakaryocytes) are anucleated cells with a large number of secretory granules that occur abunduntly in blood (300,000 cells/ul). They may approach and collide with the healthy vessel wall but will not adhere to its vascular endothelium since normal healthy endothelium is

protected by the continuous synthesis and release of antiaggregating PGI_2. However, when the endothelium is damaged, it is no longer protected. In this case the endothelial cells are either disrupted or completely removed so that the subendothelial layers, which contain proaggregatory collagen fibers and smooth muscle cells, are exposed. Platelets collide with the damaged part of a blood vessel wall and will, in the absence of PGI_2, adhere to the exposed structure. They also stick to each other and form a platelet plug; this process is called aggregation and is accompanied by secretion of the content of the granules from platelets. The content of the granules include ADP, serotonin, platelet factor 4, calcium, lysosomal enzymes, growth factor, prostaglandins, TXA_2 and 12-HETE (Moncada and Vane, 1979). Some of these substances (e.g. serotonin , TXA_2, and ADP) are platelet agonists and enhance platelet aggregation and a thrombus or hemostatic plug begins to form.

Aggregation can be measured in vitro with the aggregometer (Born and Cross, 1963). Platelet aggregation in vitro is believed to mimic the formation of the hemostatic platelet plug and platelet thrombus in vivo. The function of the platelet plug is to seal off the torn blood vessel and prevent the loss of large amounts of blood. The thrombus covers only the area of damage and does not spread up or down the vessel wall, nor does it grow across to occlude the vessel. Clearly, if the thrombus were to

enlarge significantly, then the blood flow through that vessel would be reduced and this could result in ischaemic damage to the tissues serviced. The growth or spread of the thrombus may well be limited by PGI_2 released from healthy vascular endothelial cells on either side of the damaged area and from those of the opposite blood vessel wall.

Endothelial cells of blood vessels synthesize PGI_2 from both PG endoperoxides produced in the endothelial cell and PG endoperoxides released from platelets (Bunting et al., 1976). Endothelial cells are known to convert PG endoperoxides to PGI_2 avidly, but AA to PGI_2 only feebly. Thus, in vivo it has been proposed (Moncada and Vane, 1979) that platelets close to endothelial cells are the major contributor of precursor endoperoxides from which the endothelial cells then make PGI_2. According to this theory, platelet cyclooxygenase synthesizes endoperoxides from endogenous arachidonic acid for conversion to TXA_2 which causes platelet aggregation. However, if the platelet is in close contact or approaching the vessel wall then endoperoxides are released and converted to PGI_2 by the endothelial cells of the lining. PGI_2, in turn, prevents adhesion of the platelet to the vessel wall, disaggregates loose thrombi, causes vasodilation and facilitates blood flow.

Much progress has been made in understanding the role of thromboxane and prostacyclin in hemostasis and thrombosis. It is probable that alterations in their

balance contribute to thrombotic tendencies in many circumstances. This antagonistic balance between TXA_2 and PGI_2 may be perturbed in several conditions such as excessive arachidonic acid availability (Silver et al., 1974), diabetes (Gerrard, 1985), hypercholesterolemia (Wang et al., 1988), atherosclerosis (Zmuda et al., 1977), vitamin E deficiency (Chan, 1987), and selenium deficiency (Schoene et al., 1986). For example, if the arteriole walls are coated by plaque or fatty deposits, thrombus formation is not counteracted by concurrent PGI_2 synthesis because the endothelial tissue which contains the necessary enzymes is covered and PGH_2 is not converted to PGI_2. The thrombus together with TXA_2 may result in clogging of the arteriole, thereby restricting blood flow which results in occlusion and thrombosis. When this occurs in arteries supplying the heart muscle or brain, the result is coronary thrombosis or stroke, respectively.

Clinical implications. New approaches to the treatment of diseases such as listed earlier are being sought by developing drugs that tilt the balance of these synthetic steps in favor of PGI_2, either by inhibiting thromboxane synthase, protecting prostacyclin synthase, stimulating PGI_2 production, or administering prostacyclin itself or an analog. The total effect of such intervention is the reduction of platelet activity in vivo and increased bleeding time.

In addition to administering drugs, dietary manipulation by supplementation or dietary change may provide prophylactic as well as therapeutic benefits. Knowledge of the role of dietary linoleic acid as a precursor of AA, prostaglandins, and leukotrienes has stimulated several studies of dietary manipulation as a means of selectively altering TXA_2/PGI_2 ratios and thereby reducing thrombotic tendency. Dietary linoleic acid that is converted to AA can exert a potent impact on thrombosis (Marcus, 1984; Hornstra, 1982). In contrast, feeding high levels of linoleic acid or vegetable oil has limited benefits because both PGI_2 production and TXA_2 production appear to be similarly affected, i.e. there does not appear to be a selective effect on platelet or endothelial production of TXA_2 or PGI_2 (TenHoor, et al., 1980; Hornstra, 1982; Socini et al., 1983; Ferreti et al., 1985). In fact, high intakes of n-6 fatty acids may be undesirable, as they may predispose the subject to excessive TXA_2 generation and precipitate pathophysiological states (Lands, 1986). An example of excessive essential fatty acid availability was provided by Silver et al. (1974), who showed that intravenous injection of arachidonic acid to rats produced platelet aggregation in the lung and sudden death. Similarly, when Seyberth et al. (1975) fed 6 g of arachidonate daily to healthy volunteers, they found it necessary to remove half the subjects from the study in progress because of indications of an increased tendency for

thrombosis. However, different families of unsaturated fatty acids may affect cyclooxygenase and lipoxygenase pathways differently. The unsaturated fatty acids of the n-3 family could selectively affect the different enzymes and thereby alter the thrombogenic tendency (i.e. TXA_2 production) of circulating platelets.

N-3 Fatty Acids and Arachidonic Acid Metabolism

Characteristics of Fish Oils

Epidemiological studies (Bang et al., 1976; Kromhout et al., 1985) suggested that the regular consumption of fish has a preventive effect on coronary heart disease. Many studies (Bang and Dyerberg, 1980; Herold and Kinsella, 1986; Harris, 1989) have been done to confirm the earlier studies and to investigate the biochemical evidence for the nature of the mechanisms involved. The results suggest that unique long chain polyunsaturated fatty acids (PUFAs) found in marine species are largely responsible for the beneficial effects of fish consumption though the participation of other compounds has not been ruled out (Kromhout et al., 1985; Lands, 1986). The polyunsaturated fatty acids in oils of marine origin differ considerably from those from other sources in that there are more polyunsaturated fatty acids that contain twenty or more carbon atoms in the chain. The greatest proportion of these long chain fatty acids belongs to the n-3 family. The two most common long chain fatty

acids in fish and fish oil are eicosapentaenoic acid (EPA)(20:5 n-3) and docosahexaenoic acid (DHA)(22:6 n-3) (Kinsella, 1986b). The n-3 structure allows one more double bond to be introduced into the chain than does the n-6 structure. The n-3 fatty acids (especially EPA) interfere with the metabolism of AA at several biochemical steps.

Substrate Availability

With the consumption of n-3 PUFAs, there is usually a concomitant depression of 20:4 n-6 concentration in tissue phospholipids (Herold and Kinsella, 1986; Croft et al., 1985). In order for fish oils to act physiologically, they must first be digested, absorbed, and transported to the tissue. Long chain fatty acids in fish oil triacylglycerols undergo lipolysis in the small intestine yielding free fatty acids and monoacylglycerols, both of which are readily absorbed by the enterocyte. Triacylglycerols are resynthesized from these absorbed products and assembled into chylomicrons in enterocytes. Chylomicrons are secreted into the lymphatic circulation rather than the blood as are most nutrients. They enter into the blood stream at the thoracic duct. They are cleared by the lipolytic action of lipoprotein lipases which release fatty acids from the glycerol backbone for uptake by the liver, adipose tissue and other tissues. The absorption and transport of n-3 fatty acids from fish oil is similar to that of n-6 fatty acids (Nelson and Ackman, 1988).

The major mechanism by which the consumption of fish oil reduces the level of tissue 20:4 n-6 may be via inhibition of the enzymes converting dietary linoleic acid to arachidonic acid (Kurata and Privett, 1980; DeSchrijver and Privett, 1982; Garg et al., 1988a,b). Garg et al. (1988a) demonstrated that fish oil reduced the synthesis of AA by inhibiting delta-6 desaturase, the rate-limiting enzyme in the 20:4 n-6 biosynthetic pathway, causing a depressed level of synthesis of the long chain members of the linoleate family, 20:4 n-6, 22:4 n-6 and 22:5 n-6 (DeSchrijver and Privett, 1982). Nassar et al. (1986) suggested that there is an inhibitory effect from dietary 20:5 n-3 on delta-5 desaturase which caused increased levels of 20:3 n-6 in tissues. Kurata and Privett (1980) reported that the inclusion of menhaden oil in diets depressed the delta-6 desaturase, elongase, and delta-5 desaturase. The major site of the desaturation and elongation is in hepatic microsomes. The n-3 fatty acids, EPA and DHA, are preferentially accumulated at this location (DeSchrijver and Privett, 1982). The increased concentration at this site may impact upon their ability to modify the metabolism of n-6 fatty acids.

Another mechanisms suggested for the reduction of tissue AA by fish oil feeding is the competetion between AA and EPA for incorporation into the 2-position of phospholipids. Long chain PUFAs including AA or EPA, usually occur in the C-2 position of membrane phospholipids

(Stubbs and Smith, 1984), a fact which imposes an upper limit on the amount of PUFAs that can be held in membrane phospholipids. Since the incorporation of AA and EPA into these lipids proceeds at comparable rates (Iritani and Fujikawa, 1982), they extensively substitute for each other, according to the availability of dietaty PUFA. This competition of EPA with AA for incorporation into phospholipids was observed in rat liver, plasma, heart (Iritani and Fujikawa, 1982) and platelets (Iritani and Narita, 1984; Sprecher, 1986).

Thus, fish oil consumption is accompanied by changes in the fatty acid composition of lipids throughout the body. In platelets, increases in EPA and DHA levels were noted (Iritani and Narita, 1984; Hornstra et al., 1981), although DHA was less easily incorporated into platelet phospholipids than EPA (Bruckner et al., 1984). The effects of fish oil consumption on LA and AA levels in platelets are conflicting. Morita et al. (1984) showed no change in the concentration of LA, whereas Iritani and Narita (1984) showed decreases in its concentration concomitant with decreases in the concentration of AA. Alterations in the composition of fatty acid from platelet phospholipids after dietary MaxEPA closely correlates with changes in plasma fatty acids (Croft et al., 1985). The inclusion of fish oils in the diet produced significant increases in the EPA and DHA levels in plasma lipids of rats and dogs (Croft et al., 1984; Iritani and Fujikawa, 1982; Garg et al., 1989).

The AA content of phospholipids decreased (Iritani and Fujikawa, 1982; Iritani and Narita, 1984; Garg et al., 1989) or remained essentially unchanged (Hamazaki et al., 1982). Both increases (Socini et al., 1983) and decreases (Iritani and Fugikawa, 1982) in the LA concentration in plasma lipids were noted.

Following consumption of fish oil by rats and rabbits, the EPA and DHA content of aortic phospholipids and total lipids increased by several hundred percent (Bruckner et al., 1984; Socini et al., 1983). The AA content decreased by one-fifth (Bruckner et al., 1984) or to almost one half (TenHoor et al., 1980) of its initial level. The linoleic acid level remained unaltered (Bruckner et al., 1984: Socini et al., 1983), although in other studies an increase (Socini et al., 1983) and a decrease (Lockette et al., 1982) were reported.

In livers from animals, increases in the proportions of EPA and DHA and decreases in arachidonic acid content were observed frequently (Iritani and Fujikawa, 1982; Iritani and Narita, 1984; DeScrijver and Privett, 1982; Bruckner et al. 1984; Croft, 1984). The change in linoleic acid content was more variable, i.e. increased (Kurata and Privett, 1980; DeScrijver and Privett, 1982) or decreased (Iritani and Narita, 1984; Garg et al., 1989).

Data from human feeding trials (Siess et al., 1980; Goodnight et al., 1981; Thorngren and Akesson, 1987) generally agree with the observations from animal studies.

In one human feeding trial, vonShacky et al. (1985) observed rapidly increased EPA and DHA levels in plasma phospholipids when they supplemented the diet of subjects with cod liver oil for 20 weeks. In platelet phospholipids, EPA and DHA increased slower than in plasma phospholipids, concomitantly there was a decrease in the content of AA and no change in that of LA. Erythrocyte lipid fatty acid levels were also altered in response to fish or fish oil consumption: increases in EPA were much greater than those of DHA; LA and AA showed slight decreases.

Arachidonic acid and EPA were found predominantly as esters in membrane phospholipids; only trace amounts were detected as free fatty acids (Siess et al., 1980). Little information is available concerning the direct influence on phospholipase activity by the consumption of n-3 fatty acids. Both EPA and AA are released well in response to the activation of phospholipase (Siess et al., 1980; Needleman et al., 1979). Thus, depressed phospholipid AA level and increased EPA levels will lead to an increase in released EPA and decrease in released AA.

Enzyme Activity

Cyclooxygenase and thromboxane synthase.

Eicosapentaenoic acid consumption affects eicosanoid synthesis in three ways. First, the size of the AA pools available for the synthesis of eicosanoids of the 2 series is apparently decreased by EPA consumption.

Second, in an in vitro study Needleman et al. (1979) indicated that EPA competitively inhibits the transformation of AA to TXA_2. When unlabelled EPA and ^{14}C-AA were mixed in varying ratios and the reaction was initiated by the addition of washed platelets, EPA effectively competed with AA such that a 1:1 mixture of fatty acids resulted in a 50% inhibition of formation of TXB_2, 12-HETE and HHT. Hwang et al. (1988) reported that the capacity of platelets to synthesize TXB_2 during collagen-induced aggregation was suppressed by fish oil feeding. At the present time no investigations can separate the effect of enhanced EPA levels from the effect of depressed AA levels.

Third, released EPA forms eicosanoids of the 3-series. Through the cyclooxygenase pathway, EPA produces PGG_3 and PGH_3 which are transformed further to TXA_3 by thromboxane synthase (Needleman et al., 1979; Fischer and Weber, 1983). The other stable product of the cyclooxygenase pathway is HHTE (hydroxyheptadecatetraenoic acid). TXA_3 is less active than TXA_2 in aggregating platelets (Needleman et al., 1979). EPA is a much poorer substrate for platelet cyclooxygenase (or thomboxane synthetase) than AA; it is converted one-eighth as efficiently to thromboxane (Needleman et al., 1979). Fischer and Weber (1983) showed that the amount of TXB_3 formed in platelets from human subjects receiving cod liver oil was 5-15% of the amount of TXB_2. In animal studies, immunochromatographic analysis of platelet samples indicated that feeding fish oil does not result in the

formation of significant amount of TXB_3 (Hwang et al., 1988). The amount of TXB_3 in platelets of rats fed fish oil was 1.4% of the amount of TXB_2. At the present it is unknown whether species differences exist with regard to cyclooxygenation of EPA.

Prostacyclin synthase. In in vitro systems, including rat arterial tissue (Dyerberg et al., 1981), bovine and porcine aortic microsomes (Needleman et al., 1979) and human umbilical arteries (Dyerberg et al., 1981), formation of PGI_3 from exogenous EPA or endoperoxide PGH_3 by prostacyclin synthase has been demonstrated. PGI_3 is as antiaggregatory as PGI_2 (Moncada et al., 1976b). The changes in PGI_3 and PGI_2 levels by fish or fish oil consumption were variable (Bruckner et al., 1984; Hornstra et al., 1981; Hamazaki et al., 1982) in in vivo systems. Bruckner et al. (1984) showed no changes in concentration of 6-keto-PGF_1alpha in rats fed n-3 PUFA enriched menhaden or shark liver oils. The fish oils were provided at less than 1.5g per 100g of diet (1.5 wt%), providing 2.4% of dietary energy (2.4 en%). (In animal feeding trials the level of fish oil provided is reported in one of two ways. It can be given as weight percent, wt%, which is the weight of the diet that is oil expressed as percent. It can also be given as the percent of the calories that are oil, which is called energy percent, en%. The values can be interconverted if the caloric content of the diet per unit of weight is known.) Hornstra et al. (1981) observed a 50% decrease of 6-keto-

PGF_1alpha in rats fed cod liver oil as opposed to sunflower seed oil. Hamazaki et al. (1982) reported rats fed EPA ethylester had elevated PGI_2-like activity with elevated 6-keto-PGF_1alpha in the aorta. They failed to detect significant amounts of delta 17-6-keto-PGF_1alpha, the stable metabolite of PGI_3, in rats fed EPA ethylester. Hornstra et al. (1981) observed no appreciable formation of PGI_3 in aorta of rats fed cod liver oil, whereas Fischer and Weber (1984) showed that ingestion of fish oil (4 g EPA per day) or mackerel (10-15g EPA per day) by human subjects resulted in increased excretion of the urinary metabolite of PGI_3 without suppressing that of PGI_2. It is not known whether these contradictory effects on the capacity to synthesize PGI_3 from EPA were caused by species differences in fatty acid or prostaglandin metabolism, by the results of the diverse methods used, or by the varying quantities or by the types of dietary supplementation employed.

Lipoxygenase. Various fatty acids of nutritional value may be oxygenated by platelet and/or aortic lipoxygenases and their metabolites are potentially active molecules as far as platelet functions are concerned. In platelets, 12-lipoxygenase is metabolically the most active (Lagarde, 1988). It uses EPA less effectively than AA (Boukhchache and Lagarde, 1982). However, in the presence of 20:4 n-6 or its lipoxygenase product (12-HPETE), the lipoxygenation of 20:5 n-3 increased (Boukhchache and Lagarde, 1982; Croset and Lagarde, 1985). EPA is converted into 12-

hydroperoxyeicosapentenoic acid (12-HPEPE), which is then converted into 12-hydroxyeicosapentaenoic acid (12-HEPE).

As already described in the Lipoxygenase Section in AA Metabolism, there are two arguments to explain the effect of HPETE and HETE (the hydroperoxy and hydroxy fatty acids, respectively, synthesized from AA) on platelet aggregation. One is that HPETE inhibits the synthesis of PGI_2, the antiaggregator (Turk, 1980). The other is that HPETE and HETE inhibit platelet aggregation (Aharony et al., 1982; Croset and Lagarde, 1983). These arguments could be applied to HPEPE and HEPE too. No study has been done to compare the effects of the hydroperoxy and hydroxy fatty acids from EPA to those from AA on prostacyclin synthesis. It is known, however, that 12-HEPE inhibits thromboxane-induced aggregation more potently than 12-HETE (Croset and Lagarde, 1983). In rats fed a diet containing fish oil the formation of 5- and 12-HETE by platelets was suppressed (Hwang et al., 1988). In contrast, the formation of 12-HEPE in the group receiving fish oil was greater than in the control group (Hwang et al., 1988). A study by Hornstra et al. (1981) showed that platelets of rats fed diets containing cod liver oil produced 75% less cyclooxygenase products (HHT and HHTE) and 50% less lipoxygenase products (HETE and HEPE) than platelets of rats fed diets containing sunflower seed oil when stimulated with collagen. HHT and HHTE are considered to reflect TXA_2 and TXA_3 formation. The production of HEPE was 25% of the total products produced by the lipoxygenase

pathway while TXA_3, measured as HHTE, was not formed in significant amounts in the platelets of animals fed cod liver oil. Collectively these studies suggest that EPA released from platelet membrane lipid is preferentially diverted to the lipoxygenase pathway and that the formation of cyclooxygenase-derived products by fish oil is reduced.

Lipid Peroxide Production

Various lipid peroxides and their metabolites (e.g. endoperoxides, HPETE, HPEPE, HETE, HEPE, and MDA) can be generated by the enzymes (e.g. cyclooxygenase, peroxidase, and lipoxygenase) involved in the synthesis of eicosanoids and by nonenzymatic reactions that can occur to these compounds. Because eicosanoid production is highly dependent on the peroxide tone, balancing the production and destruction of lipid peroxides is important for maintaining health, specifically as it relates to the preferred balance of prostacyclin and thromboxane.

High PUFA consumption is associated with lipid peroxidation as well as eicosanoid synthesis. Replacing dietary saturated fats by vegetable oils and/or fish oil leads to increases in the content of PUFAs in tissue which are more susceptible to lipid peroxidation. These processes generate free radicals. In addition to the generation by lipid peroxidation and/or by the activity of cyclooxygenase and lipoxygenase, free radicals originate from the electron transport system and from the activity of various oxidases. They can also be introduced exogenously as components of

tobacco smoke and air pollutants and indirectly through the metabolism of drugs and pesticides. Free radicals such as peroxy radicals, the superoxide anion, and the hydroxyl radicals can cause the change of eicosanoid synthesis as mentioned earlier and they can also cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes, nucleotides in DNA and sulfhydryl bonds in proteins. In addition, certain aldehydes such as MDA and hydroxynonenal, arising from the free radical degradation of PUFAs, can cause cross-linkings in lipids, proteins, and nucleic acids. There is some evidence that free radical damage contributes to the etiology of many chronic health problems such as cardiovascular disease, inflammatory process, and cancer (Machlin and Bendich, 1987).

One of the currently available methods to measure lipid peroxides is the thiobarbituric acid test which measures malondialdehyde (MDA), one of the degradation products. Malondialdehyde formation also correlates with the production of endoperoxides and thromboxanes from AA (TenHoor et al., 1980; Hornstra et al., 1981), thus it has been used as an alternative method for the measurement of thromboxane production in platelet. But the measurement of MDA in stimulated platelets is not the method of choice for thromboxane because it is not specific. It is still appropriate, however, for establishing lipid peroxidation status. There are contradictory effects of fish oil on MDA production (Hornstra et al., 1981). Its production in

collagen-stimulated platelets from rats consuming diets rich in cod liver oil was decreased compared to synthesis in platelets from rats consuming a corn oil rich diet (Hornstra et al., 1981). Increases in MDA production in AA treated and N-ethylmaleimide treated human platelets following consumption of fatty fish or fish oil have been reported (Brox et al., 1981; Goodnight et al., 1981). Like collagen, both AA and N-ethylmaleimide have been thought to stimulate thromboxane synthesis. When MDA was measured in plasma and liver in rats, as opposed to in PRP used during aggregation, in fish oil fed groups its production was higher than in corn oil fed groups (Panganamala et al., 1989; Kobatake et al., 1983). In NZW-rabbits fed diets containing either fish oil, corn oil or lard for 12-weeks, plasma MDA was 3 times higher in the fish oil group than the other groups (Panganamala et al., 1989). In the experiment by Kobatake et al. (1983), a PUFA mix was prepared from liver oil from squids in which the n-3 type fatty acids consisted of 75 percent of the total fatty acids. Lipid peroxides in the liver and serum were more elevated in rats fed diets containing 1, 3 and 5 wt% PUFA mix than in rats fed diets containing 5 wt% oleate or 5 wt% linoleate. Increases in the production of lipid peroxides proportional to the amount of PUFA mix consumed were observed when rats fed the PUFA mix alone or the mix combined with varying proportions of methyl oleate were compared to those fed lard. Lipid peroxide (measured as umol MDA/100g liver) was increased 51%

in rats fed a squid liver PUFA mix rather than methyl oleate (Kobatake et al., 1983). To date, no study has been done to investigate the effect of fish oil on MDA level in aorta.

Balance between the Production of Thromboxane and Prostacyclin

By the mechanisms described above, fish oil is known to reduce selectively TXB_2, shifting the balance between thromboxane and prostacyclin in favor of prostacyclin. The quantity of fish oil in the diet seems to control the decrease of TXA_2 without decreasing significantly the amount of PGI_2 (German et al., 1985). German et al. (1985) observed that in the presence of adequate linoleic acid - i.e. 2.5 en%- serum TXA_2 appeared to be depressed to a greater extent than PGI_2 (i.e. a favorable antithrombotic status) at low dietary levels of fish oil (<5 wt%); whereas at higher dietary levels of fish oil (10, 20 wt%) both PGI_2 and TXA_2 were equally reduced. Similarly, even in the presence of inadequate LA, Croft et al. (1984) observed a significant drop in serum TXA_2 compared to aorta PGI_2 at low dietary levels (5 en% cod liver oil); whereas both were significantly depressed at higher (20, 40 en%) intake of cod liver oil.

As discussed earlier an altered balance between the synthesis of thromboxane and prostacyclin may be associated with changes in platelet aggregation, thrombotic tendencies and bleeding time. The use of fish and fish oils in diets may provide a way in which this balance can be controlled.

In both laboratory animals (VasDas et al., 1982) and humans (Fischer and Weber, 1983,1984; Thorngren and Gustafson, 1981: Thorngren et al., 1984), reductions in ADP- and collagen-induced platelet aggregation have been observed following the experimental fish diets or supplement with fish oils. The decreased aggregation was proportional to the extent of EPA incorporation into platelet phospholipid in rats (Morita et al., 1984). In contrast, there have also been disagreements on the antiaggregating effects of fish oil (Bruckner et al., 1984). In a study in rats there was no significant difference in platelet aggregation between the group of animals fed diets containing fish oil and the group fed control diet containing triolein and safflower oil. In a human study salmon oil supplementation in the diet showed an effect on aggregation with ADP but not collagen (Goodnight et al., 1981). When 12 males were maintained for 6 weeks on a fish diet, aggregation stimulated by ADP and a maximal dose of collagen (10 ug/ml) was decreased throughout the diet period and for several weeks after the end of the diet, while the fatty acid composition of platelet and plasma phospholipids showed an increase in n-3 and decrease in n-6 fatty acids but returned to pretreatment values after the end of diet (Thorngren et al., 1984). In addition to the reduction in platelet aggregation, arterial thrombotic tendencies decreased with fish oil supplementation. A longer obstruction time of a cannula inserted into the abdominal aorta was measured in

rats consuming diets supplying 45 en% from cod liver oil and 5 en% from sunflower oil when compared to only 5 en% from sunflower oil (Hornstra et al., 1981).

Bleeding time, measured as the time required for blood flow from the right saphenous vein through a syringe needle to stop, did not increase significantly in rats fed diets containing either 1.5 wt% n-3 PUFA enriched menhaden oil (about 2.4 en%) or 1.09 wt% n-3 PUFA enriched shark liver oil (about 1.7 en%)(Bruckner et al., 1984). In contrast, rats fed a diet with 45 en% cod liver oil showed a 52% increase in bleeding time when the tip of the tail was transected and immersed in warm saline (Hornstra et al., 1981). The observations in these studies could differ not only from the different proportions of fish oil in the diet but could also be related to the different body sites and method used to measure bleeding time.

In human feeding trials (Goodnight et al., 1981; Thorngren and Gustafson, 1981; Sanders and Roshanai, 1983), on the other hand, nearly a 50% increase in bleeding time was observed after the consumption of fatty fish. Hemostatic effects caused by dietary fish cannot always be accounted for by decreased platelet aggregability (Sanders and Roshanai, 1983; Thorngren and Gustafson, 1981) or by the increase of n-3 fatty acids in the platelets. It depends not only on platelets and their capacity to produce thromboxane but also on the reactivity of injured vessels and on the plasma coagulation mechanism.

Selenium and Arachidonic Acid Metabolism

Most of the current research on the possible beneficial effects of seafoods seems to be directed at the effects of 20:5 n-3 and/or 22:6 n-3 fatty acids in fish oil. Several researchers have suggested that perhaps the beneficial effects are due to a combination of fatty acids and/or some component present in trace amounts, and that too much purification might result in the removal of this factor (Lands, 1986). One suggested factor is selenium which is present in fish in high concentrations (0.4-0.7 ug/g)(Morris and Lavender, 1970) compared to the amounts that are found in other foods. Though organ meats contain 1.5 ug/g, most meats contain 0.2-0.5 ug/g. Grain and cereal products vary widely containing from 0.025 to 0.66 ug/g, the amount found depending on soils or processing. Dairy products contain 0.07 ug/g and fruits and vegetables contain less than 0.01 ug/g. The following section provides a biochemical basis for the beneficial effect of selenium on cardiovascular disease.

Characteristics of Selenium

Several studies (Bryant and Bailey, 1980; Masukawa et al., 1983; Schoene et al., 1986; Funk et al., 1987) suggest that selenium is associated with the metabolism of AA at different sites and may exert an antithrombotic effect on platelet and blood vessel wall functions. Before considering the specific mechanisms of alteration of AA

metabolism by dietary modulation of selenium, it is necessary to review briefly some of the characteristics of selenium.

Selenium is an essential mineral for humans and animals according to the definition of essentiality by Mertz (1981): a nutrient is considered essential if its deficiency consistently results in the impairment of a function, reducing it from optimal to suboptimal. Selenium is an essential constituent of glutathione peroxidase (EC 1.11.1.9), an enzyme which is located in the cytosol and mitochondrial matrix and protects tissues against peroxidation by reducing H_2O_2 or hydroperoxides (organic hydroperoxy acids) to H_2O and hydroxy fatty acids (Rotruck et al., 1973). In addition to this protective enzyme, there is the GSH-S-transferase which also possesses peroxide-reducing activity, although it acts on lipid peroxide and not on H_2O_2. This non-selenium enzyme is found in cytosolic and microsomal fractions and is designated selenium-independent glutathione peroxidase activity. Activities of some of GSH-S-transferases increase when dietary selenium is inadequate as a compensatory mechanism for the maintenance of non-lethal levels of hydroperoxides within the cell (Lawrence et al., 1978). However, the experiment by Burk et al. (1980) showed that GSH-S-transferase was not elevated in selenium-deficient female, castrated male, or testosterone-treated female rats even though the activity of the selenium-dependent GSHPx was depressed in all groups.

Therefore, the hypothesis that the increased GSH-S-transferase activity observed in selenium-deficient male rats is due to decreased selenium-dependent GSHPx activity is in doubt. However, some tissues such as platelets, heart, lung and skin, possess little or no selenium independent peroxidase activity and therefore would be more susceptible to the alteration in hydroperoxide levels when GSHPx activity is either low or absent (Carmagnol et al., 1983). Homeostasis of selenium is maintained primarily by regulation of urinary excretion (Burk, 1976). Dietary requirements of most animals for selenium appear to fall in the range of 0.05 to 0.3 mg/kg dry matter. Acute selenium toxicity results in garlic breath, vomiting, dyspnea, tetanic spasm and death from respiratory failure. Chronic toxicity results in growth failure. Chronic exposure to high intakes of selenium in seleniferous areas of Venezuela and South Dakota has been associated with chronic dermatitis, loss of hair, and discoloration or brittleness of fingernails (Burk, 1976). To serve as a warning against a marginal and excessive intake of selenium, the Food and Nutrition Board of the National Research Council made a provisional recommendation that Americans ingest between 50 and 200 ug/day (FN Board, 1980).

Optimal activity of GSHPx requires an adequate dietary supply of selenium. The activity of GSHPx is low in the liver, platelet, plasma and aorta of selenium-deficient animals (Masukawa et al., 1983; Schoene et al., 1984, 1986;

Funk et al., 1987) and low in plasma and platelets in selenium-inadequate humans (Levander et al., 1981). For example, GSHPx activity in platelets from rats fed a selenium-deficient diet for six weeks decreased to 13% of the activity in the platelets from control rats, whose diets were supplemented with selenium (Bryant and Bailey, 1980).

Several dietary factors are known to influence GSHPx activity. Mutanen and Mykkanen (1984) showed that the type of dietary fat (butter, olive oil, corn oil, and sunflower oil) can affect the plasma GSHPx levels in chicks without altering intestinal absorption of selenite. When the diets contained 20 wt % fat, the plasma GSHPx levels were not affected; however, when they contained 4 wt % fat, GSHPx increased with increasing proportions of polyunsaturated fatty acids in the diets. Schoene et al. (1986) showed, in rats given a diet that contained fish oil and lard, liver GSHPx was lower than in rats given diets containing corn oil. They claimed that the decreased value of GSHPx was due to the lack of linoleic acid in the diet instead of the presence of fish oil in the mixture.

Decreased GSHPx activity has been reported in animals fed diets deficient in Vitamin E or essential fatty acids (Jensen and Clausen, 1981). On the other hand, the activity of this enzyme appears to be increased in response to oxidant stress (Ganther et al., 1976). Vitamin E can protect against many of the symptoms of selenium deficiency and vice versa (Ganther et al., 1976). These sparing as

well as synergistic actions are thought to result from the ability of both tocopherol and selenium dependent GSHPx to decrease the production of lipid peroxides. Deficiency of either selenium or vitamin E causes an increased lipid peroxidation.

Substrate Availability

There are only a few studies that address the effects of selenium deficiency on tissue fatty acid composition. Therefore, this review was expanded to include effects of both selenium deficiency and vitamin E deficiency on tissue fatty acid composition. This extension is appropriate because both selenium and vitamin E work as part of the antioxidant defense system (Machlin and Bendich, 1987). Vitamin E, a lipophilic molecule, is a scavenger of free radicals. On reaction with fatty acid peroxyl radicals in cell membranes, vitamin E is oxidized to a tocopherol semiquinone radical, which is rapidly degraded, and the original free radical receives a hydrogen atom, thereby producing fatty acid hydroperoxide. As described earlier selenium plays a role in decreasing lipid peroxidation through the enzyme GSHPx, which converts lipid hydoperoxide or hydrogen peroxide into H_2O and hydroxyl fatty acid. For optimal function of GSHPx, reduced glutathione (GSH) is required. Sulfur-containing amino acids (cysteine or methionine) provide cysteine for the synthesis of the tripeptide glutathione (L-glutamyl-L-cysteinyl glycine). To date, no work has been done to investigate the effect of

antioxidant deficiency on the incorporation of fatty acids into tissue lipids such as phospholipids. No study has been done to investigate the direct effect of selenium deficiency on phospholipase A_2 activity although Toivanen has suggested that selenium deficiency may increase its activity (Toivanen, 1987). Vitamin E deficiency is known to increase its activity (Panganamala and Cornwell, 1982). The available studies were usually performed to see the change induced in the tissue fatty acid composition of tissues after antioxidant deficient diets were fed for long periods of time.

Effect of antioxidant deficiency on the level of AA. The reported effects of antioxidant deficiency on AA levels in tissues are contradictory. The susceptibility of fatty acids to peroxidation increases with the increase in the number of double bonds in the molecule (Witting, 1967). The amount of arachidonic acid in heart, plasma and liver was lower in antioxidant deficient chicks, rats (Bieri and Andrew, 1964) and lambs (Pendell et al., 1969). In another work, Bieri and Andrew (1963) failed to see any changes of fatty acids composition of liver and plasma due to selenium deficiency. On the other hand, Horwitt et al. (Horwitt, 1965; Witting, 1967; Witting et al., 1967) observed that in the skeletal muscle, liver and testes the level of AA was increased over normal levels when vitamin E, selenium and methionine were deficient. Fischer and Whanger (1977) also

showed increased AA in the liver of selenium deficient rats compared to selenium-supplemented ones.

Effect of antioxidant deficiency on the level of n-6 fatty acids. The other members of the n-6 family of fatty acids are changed in a variable manner. The testes of the vitamin E-deficient (19-28 week) rats had increased proportions of 18:2 n-6 and 22:4 n-6, decreased amounts of 22:5 n-6 (Bieri and Andrew, 1964; Willms et al., 1987) but no change in the proportion of 20:3 n-6. Witting and Horwitt (1967) showed a decreased percentage of linoleic acid in skeletal muscle of vitamin E deficient rats.

Effect of antioxidant deficiency on the level of EPA and DHA. In the study by Witting and Horwitt (1967) rats were fed diets deficient in selenium, methionine, and vitamin E containing 12.5 wt% fat for a long period (23-30 weeks). The fat was specially prepared to be highly unsaturated. In hepatic phospholipids, EPA was significantly decreased. In skeletal phospholipids from the antioxidant-deficient rats, the percent of DHA was decreased.

Enzyme Activities

Elevated levels of hydroperoxides due to low GSHPx activity (Bryant and Bailey, 1980; Masukawa et al., 1983; Guidi et al., 1986) could unfavorably alter the balance among eicosanoids produced by various cells resulting in aberrant responses to noxious stimuli.

Cyclooxygenase and thromboxane synthase. The role of selenium or GSHPx in cyclooxygenase and thromboxane synthase is unclear. Low GSHPx activity might increase the level of peroxides but still maintain them lower than the 15 umol/l level which is known to impair cyclooxygenase and probably thromboxane synthase activity. Kawaguchi et al. (1982) showed increased TXB_2 synthesis accompanied lowered GSHPx activity in the microsomal fractions of platelets of rabbits fed a hypercholesterolemic diet, a diet which is supposed to increase lipid peroxides. Schoene et al. (1986) showed that collagen-stimulated platelets from rats fed a selenium-deficient diet for 12 weeks produced increased amounts of TXB_2 as measured by radioimmunoassay when compared to platelets from control rats. The GSHPx activity of platelets from patients with coronary heart disease (Guidi et al., 1986) is lower than normal and mean MDA production was increased in the patient group.

Prostacyclin synthase. In vascular microsomes and in fresh vascular tissue incubated separately or in platelet rich plasma, 15-HPETE strongly and selectively inhibited prostacyclin synthase, the enzyme responsible for the formation of prostacyclin from endoperoxides (Gryglewski et al., 1976). Vascular prostacyclin synthase activity was inhibited by 12-HPETE (Turk, 1980). Other peroxides of fatty acids and their methyl esters were also strong inhibitors of prostacyclin synthase (Salmon et al., 1978). Thus, accumulated 12-, 15-HPETE , and other lipid peroxides

due to selenium deficiency could potentially inhibit prostacyclin synthase.

According to Moncada and Vane (1979), prostacyclin synthase is much more sensitive to peroxide levels than cyclooxygenase and thromboxane synthase. A peroxide concentration of 1.5 umol/l results in inactivation of prostacyclin synthase whereas it can still activate cyclooxygenase and thromboxane synthase. Kent et al. (1983) also demonstrated that cyclooxygenase and prostacyclin synthase were differently inhibited in a dose-dependent manner by hydroperoxide (15-HPETE).

Wang et al.(1988) reported that high levels of lipid peroxides were found in atherosclerotic aorta, especially in plaque regions. GSHPx activities were decreased in atherosclerotic rabbit plasma and plaque regions of aorta compared to controls. The PGI_2 production in the plaque regions was lower than in nonplaque regions of atherosclerotic aorta. Control aorta PGI_2 production was higher than in the atherosclerotic aorta. It has been reported that prostacyclin synthesis is reduced and MDA levels increased in aorta from selenium-deficient rats (Masukawa et al., 1983). Aorta from selenium-deficient rats produced significantly less PGI_2 compared to controls as determined by bioassay based on the inhibition of platelet aggregation (Schoene et al., 1986). When added to the culture medium of porcine aortic endothelial cells, selenium also increased prostacyclin production (Schiavon et al.,

1984). Addition of selenium to a normal diet has been shown to increase prostacyclin synthesis by aorta (Doni et al., 1983).

Lipoxygenase. GSHPx may be involved in the metabolism of 20 carbon fatty acids in the lipoxygenase pathway at two points. GSHPx may suppress lipoxygenase activity. For example, a 12-lipoxygenase from rat lung is sensitive to GSHPx (Yokoyama et al., 1983) and 5-lipoxygenase is inhibited by GSHPx (Egan et al., 1983). Thus, decrease of HPETE production may occur in the presence of GSHPx. In addition, GSHPx is involved in the reduction of 12-HPETE to 12-HETE in platelets (Bryant and Bailey, 1980). Selenium deficiency in rats causes 12-HPETE to accumulate, resulting in its conversion to epoxyhydroxy and trihydroxy compounds by an alternate pathway. 15-HPETE produced from AA by 15-lipoxygenase also accumulates in selenium-deficient animals. Increased HPETE would inhibit prostacyclin synthase as described in the above section.

Balance between the Production of Thromboxane and Prostacyclin

As just discussed, selenium deficiency can inhibit prostacyclin synthase by increasing the generation of hydroperoxides (e.g. 12- or 15-HPETE) and thus enhance the inhibition of PGI_2 synthesis and/or it can increase TXA_2 synthesis also by increasing the generation of hydroperoxides (e.g. PGG_2) above the level needed to

activate cyclooxygenase and TXA_2 synthetase. Thus, selenium deficiency should tip the balance of thromboxane and prostacylin toward the proaggregatory state (Masukawa et al., 1983; Schoene et al., 1986; Wang et al., 1988). Masukawa et al. (1983) showed increased aggregation in response to ADP, collagen and AA in platelets from selenium-deficient rats compared to platelets from selenium-adequate rats.

Schoene et al. (1984, 1986) showed that selenium-deficient, gel-filtered rat platelets aggregated to a greater extent when stimulated with collagen and ADP than control platelets. In work done by Schiavon et al. (1984) bleeding time doubled in a group of human volunteers after a 6-week treatment: daily oral administration of selenium as an aqueous solution of sodium selenite (10 ug/kg of body weight). A strong association between low serum selenium and enhanced platelet aggregation was observed in the Kupio Ischaemic Heart Disease Risk Factor Study (KIHD) by Salonen and the study group in Finland (Salonen, 1989). Wang et al. (1988) reported that the PGI2 production in aortic plaque was less than in atherosclerotic non-plaque tissue which, in turn, was less than in healthy aorta. The rates of TXA_2 production were in the reverse order. These results provide supportive data in favor of the association between atherogenesis, elevated lipid peroxides, and disturbances in PGI_2/TXA_2 balance.

Although both selenium and EPA have been shown to be involved in eicosanoid synthesis, thrombosis and heart disease, no studies have attempted to investigate the antiaggregatory effects of the combination of these two nutrients under controlled conditions. Selenium might synergistically influence prostaglandin synthesis in the presence of high EPA. However, in most studies when EPA was the focus of a study, the level of selenium in the diet was not considered; and conversely when dietary selenium was emphasized, the EPA level in the diet was not considered. There have been a few exceptions to this. For instance, Schoene et al. (1986), in a study designed to evaluate the role of selenium on metabolism of AA, suggested that the type of fat influenced the effect of selenium supplementation. However, they emphasized the adequacy of linoleic acid without questioning the role of EPA. Their study was marred by noncomparable diets. One diet provided 5 wt% corn oil; the other 8 wt% of fat. This second diet was a combination of lard and cod liver oil at the ratio of 5:3 and contained insuffient essential fatty acids. The first diet, on the other hand, had adequate levels of essential fatty acids. In addition, the first diet contained a lower percentage of the calories as fat than did the second diet. One study in humans has tried to evaluate both selenium and EPA. Thorngren and Akesson (1987) fed 150-200 g of fatty fish, mainly herring, salmon and mackerel, per day to healthy Swedish individuals as part of

their normal diet. The fish provided an additional 40-50 ug of selenium in addition to supplying 2-3 g of EPA per day. The increase in plasma selenium persisted throughout the fish diet period and for several weeks thereafter. The changes in the fatty acid composition of platelets and plasma such as an increase in EPA and a decrease in AA occurred within a week and persisted throughout the diet and reversed to pretreatment values after fish was withdrawn from the diet. The changes in the fatty acid compostion of platelet and plasma did not run the same time course as the hemostatic parameters: the bleeding time prolongation due to the fish diet occurred only after 6 weeks and persisted 3 weeks after the end of the diet; the aggregation by ADP was decreased throughout the diet and for several weeks thereafter, even when the fatty acid composition had returned to normal. Due to this mismatch of changes in fatty acid profile with bleeding time and platelet aggregation, Thorngren and Akesson (1987) emphasized the antiaggregatory effect of selenium rather than the effect of EPA.

The present study will investigate the interaction of two nutrients (n-3 fatty acids and selenium) on eicosanoid synthesis and hemostatic functions (platelet aggregation and bleeding time).

MATERIALS AND METHODS

Animals

Fifty-six, 30-day-old, male Sprague-Dawley albino rats were purchased from Charles River Laboratories, Wilmington, Massachusetts. They were individually housed in suspended stainless, steel cages in a temperature-controlled animal room with a 12 hour light cycle. Rats were given distilled-deionized water. The rats were divided into four groups and fed one of four diets. The diets, which contained either low or high levels of selenium and MaxEPA or corn oil, were fed to the rats for eight weeks.

Diets

The composition of the four diets used in the study is shown in Table 2. The diet of the animals in Groups 3 (+SeCO) and 4 (+SeFO) contained about 0.4-0.5 mg of selenium as sodium selenite per kg of diet. This represents a nutritionally generous but nontoxic amount of selenium (Schoene et al., 1986). The diet of the animals in Groups 1 (-SeCO) and 2 (-SeFO) was selenium deficient. It contained less than 0.005 mg Se/kg diet. The selenium content was verified by analyzing the diets. Torula yeast was used as protein source instead of casein because both the content and bioavailability of selenium in torula yeast is very low (Schwarz, 1961).

Table 2

Composition of the diets (g/kg)

	Group 1 (-SeCO)[1]	Group 2 (-SeFO)[1]	Group 3 (+SeCO)[1]	Group 4 (+SeFO)[1]
Torula Yeast	300	300	300	300
Sucrose	540	540	540	540
Corn oil[2]	70	15	70	15
MaxEPA[3]	-	55	-	55
DL-Methionine	3	3	3	3
Mineral mix (-Se)[4]	35	35	35	35
Se as sodium selenite	-	-	0.5mg	0.5mg
Vitamin mix (-Vit.E)[5]	10	10	10	10
DL-alpha-tocopheryl acetate[6]	0.17	-	0.17	-
Alphacel	40	40	40	40
Choline bitartrate	2	2	2	2

[1]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.

[2]Mazola corn oil (Gift from Best Foods, CPC International Inc. Englewood Cliffs, NJ)

[3]MaxEPA (Gift from R.P. Scherer of North America, Troy, MI)

[4]AIN-76 mineral mix modified without selenium (TD80313) Teklad, Madison, WI; g/kg of mix: calcium phosphate dibasic, $CaHPO_4$, 500; sodium chloride, NaCl, 74; potassium citrate monohydrate, $K_3C_6H_5O_7 \cdot H_2O$, 220.0; potassium sulfate, K_2SO_4, 52.0; magnesium oxide, MgO, 24.0; manganous carbonate, $MnCO_3$, 3.5; ferric citrate, $FeC_6H_5O_7$, 6.0; zinc carbonate, $ZnCO_3$, 1.6; cupric carbonate, $CuCO_3$, 0.3; potassium iodate, KIO_3, 0.01; chromium potassium sulfate, $CrK(SO_4)_2 \cdot 12H_2O$, 0.55; sucrose, finely powdered, 118.04

[5]AIN-76A vitamin mix modified without vitamin E (TD 84127) Teklad, Madison, WI; g/kg of mix: thiamin HCl,0.6; riboflavin, 0.6; pyridoxine HCl,0.7; niacin, 3.0; calcium pantothenate, 1.6; folic acid, 0.2; biotin, 0.02; vitamin B_{12} (0.1% trituration in mannitol), 1.0; dry vitamin A palmitate (500,000 IU/g), 0.8; vitamin D_3 trituration (400,000 IU/g), 0.25; menadione sodium bisulfite complex, 0.15; sucrose, 981.08

[6]DL-alpha-tocopheryl acetate, powder, 500 IU/g, Teklad, Madison, WI

Corn oil and MaxEPA were used as fat sources in the diets. Corn oil is a source of n-6 fatty acids and was a gift from Best Foods (Englewood Cliff, New Jersey). MaxEPA is a commercially available, concentrated source of n-3 fatty acids, especially EPA, and was a gift from R.P. Scherer of North America (Troy, Michigan). The Group 2 (-SeFO) and Group 4 (+SeFO) diets contained 15 g corn oil and 55 g MaxEPA in lieu of 70 g corn oil. The 15 g corn oil per kg of diet provided sufficient linoleic acid to meet the essential fatty acid requirement.

These two fat sources contain different levels of vitamin E. Corn oil contains approximately 0.46 IU/g of oil whereas MaxEPA contains 2 IU/g of oil. According to the manufacturer's specifications, corn oil contains alpha-, gamma-, and delta-tocopherol at 0.20 mg, 1.08 mg and 0.03 mg per g of corn oil, respectively. The approximate relative biogical potencies of the isomers of tocopherol compared to the alpha form are gamma, 10% and delta, 1% (Farrell, 1988). Thus, the 1.08 mg of gamma-tocopherol in the corn oil is equal to 0.11 mg of alpha-tocopherol. The distribution of delta-tocopherol is too small to be included. One mg of d-alpha-tocopherol gives 1.49 IU of vitamin E activity. Thus, 1 g of corn oil has 0.46 IU vitamin E activity (0.46 IU is derived from 0.2 mg x 1.49 IU + 0.11 mg x 1.49 IU). According to communication with the personnel at R.P. Scherer, 2 IU of tocopherol are routinely added to fish oil to protect it from oxidation. Although the specific form of

vitamin E added to MaxEPA was not provided by the manufacturers, usually the form added to foods is dl-alpha-tocopheryl acetate. Thus, we assumed they added 2 mg of dl-alpha-tocopheryl acetate. Because 1 mg of dl-alpha-tocopheryl acetate gives 1 IU of vitamin E activity, 1 g of fish oil contained 2 IU of vitamin E activity. Because vitamin E is also known to affect prostaglandins and peroxidation of lipids, its content in all diets was adjusted to similar levels. The MaxEPA diet contained 117 IU of vitamin E per kg diet: 110 IU from 55 g of MaxEPA oil and 7 IU from 15 g of corn oil. The corn oil diets contained only 32 IU of vitamin E per kg of diet without the addition of extra vitamin. To match vitamin E levels, 0.17 g of vitamin E powder, which contained 500 IU dl-alpha-tocopherol acetate/g and provided 85 IU of vitamin E, was added to the corn oil diets. Though 117 IU of vitamin E is greater than the requirement of the rat (50 IU/kg diet) proposed by the Council of the American Institute of Nutrition (1977), higher vitamin E levels may be better in fish oil diets to prevent the increase of lipid peroxidation in the diets or to prevent the decrease of vitamin E in the body as indicated by plasma vitamin E levels (Fritsche and Johnston, 1988; Mouri et al., 1984).

The fatty acid composition of diets is shown in Table 3. It was determined by analyzing the fatty acid profile in the corn and fish oil rather than directly analyzing the fatty acid profile in the extracted lipid from

Table 3

Fatty acid composition of diets

fatty acid	Diets[1] Corn oil (-SeCO & +SeCO)	Fish oil (-SeFO & +SeFO)
	wt % [2]	
14:0	-[3]	5.93
15:0	-	0.50
16:0	10.16	17.16
16:1 n-7[4]	0.09	6.96
17:0	-	0.40
18:0	1.77	3.07
18:1 n-9	27.59	10.55
18:1 n-7	-	4.85
18:2 n-6t[5]	-	1.93[6]
18:2 n-6c[5]	58.46	13.51
18:3 n-6	-	-
20:0	0.47	0.50
18:3 n-3	1.02	0.79
20:1 n-9	0.32	1.24
18:4 n-3	-	2.79
21:0	-	0.33
20:2 n-6	-	0.14
20:3 n-6	-	0.11
22:0	0.13	0.13
20:4 n-6	-	0.84
22:1 n-11	-	0.44
22:1 n-9	-	0.21
20:5 n-3	-	14.36
22:4 n-6	-	-
24:1 n-9	-	1.04
22:5 n-6	-	0.40
22:5 n-3	-	1.73
22:6 n-3	-	10.10
total n-6	58.46	15.00[6]
total n-3	1.02	29.77
n-3/n-6	0.02	1.98

[1]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.
[2]Weight % of identified fatty acid methyl esters.
[3] - means the fatty acid was not detected.
[4]Fatty acids are denoted by their carbon chain length; the first number after the colon is the number of double bonds and the number after n indicates the location of the first double bond beginning from the methyl end.
[5]C denotes cis-isomer and t denotes trans-isomer.
[6]Trans fatty acids are not included in the category of total n-6 class.

diets. The fatty acid is identified in the same way as the plasma fatty acids, by a technique which will be described later. From the relative weight percent composition of the oils, the fatty acid composition of the diet was estimated. This estimation was based on the fact that there were 70 g of corn oil in each kg of diet in the corn oil diets and the fish oil diets contained 15 g of corn oil and 55 g of fish oil. The diets were mixed and stored in plastic containers at -20^{o}C to prevent lipid peroxidation. They were replaced daily and all unconsumed material was discarded. To assure that there was no significant increase in lipid peroxidation in the diets, the lipid peroxide content was estimated by measuring the content of malondialdehyde (MDA) as the thiobarbituric acid-reacting substance (Ke et al., 1984). In this method, MDA is distilled from the sample matrix. Distillation provides a clear solution in which MDA is measured by a method that is very similar to one used in tissue MDA measurement. This method will be described later. The MDA content of the diets was measured when they were freshly prepared, after holding them for 24h at room temperature and after holding them for one week at -20^{o}C. This mimics the conditions under which the diet was used. There was no difference in the level of MDA in the diets due to different storage conditions and length of storage.

Analytical Methods

Bleeding time. To assess the effects of various dietary regimes on the hemostatic status of the animals, the tail bleeding times were measured two weeks before sacrificing the rats for further analysis. Nine rats per each group were randomly selected. After anesthetizing an animal its tail was transected 3mm from the tip. The distal 5 cm of the tail were then immersed vertically in saline at 37.5°C. The period between transection and the moment bleeding stopped was taken as the bleeding time (Hornstra, et al., 1981; Gordon and O'Dell, 1980).

Blood and tissue preparation. After eight weeks on their respective diets, the rats were weighed, anesthetized with ketamine hydrochloride (60 mg/kg body weight) (trade name, Vetalar; Parke-Davis, Division of Warner-Lambert Co., Morris Plains, New Jersey) and xylazine (10 mg/kg body weight) (trade name, Rompun; Mobay Corporation, Shawnee, Kensas). The blood from 8-9 rats of each group were drawn by cardiac puncture into a Vacutainer tube containing sufficient 3.8% sodium citrate to yield a final 0.5 to 4.5 citrate to blood volume. The citrated blood was gently mixed and centrifuged at 200 x g for 10 min to prepare the platelet rich plasma (PRP). A Beckman TJ-6R table top centrifuge, equipped with a TH-4 rotor, was used (Beckman Instrument Inc., Palo Alto, California). The residual blood was further centrifuged at 1,800 x g for 15 min to obtain platelet poor plasma (PPP). After counting the number of

platelets in PRP using phase contrast microscopy, its platelet count was adjusted to 300,000/mm^3 with autologous PPP for platelet aggregation.

The blood from the remaining rats of each group was collected in heparinized Vacutainer tubes by cardiac puncture. After centrifugation at 4^oC, plasma was collected for later analysis of the fatty acid profile (Morrison and Smith, 1964), selenium concentration (Brown and Watkinson, 1977), GSHPx activity (Deagen et al., 1987; Paglia and Valentine, 1967) and lipid peroxide content (Satoh, 1978).

The aorta from 7 rats of each group was removed. A portion of the aorta (1.5cm) was incubated for 30 min in 1 ml of HEPES (N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid). The aortic strip was removed at the end of the 30 min-incubation, the incubated medium was stored at -20^oC to be used in the determination of the synthesis of 6-keto-PGF_1alpha (the inactive metabolite of prostacyclin) by radioimmunoassay (Mitchell et al., 1988). The remainder of the aorta was divided into two sections, frozen at -20^oC, and used in subsequent assays: one portion for lipid peroxide analysis and one for fatty acid profile. The aorta from the remaining rats of each group were removed, frozen, and stored at -20^oC for subsequent GSHPx analysis (Funk et al., 1987).

The liver was removed, weighed, frozen in liquid nitrogen and stored at -20^oC for GSHPx activity, Se concentration, lipid peroxides, and fatty acid analysis.

The heart was removed, weighed and frozen in liquid nitrogen and stored at -20°C. No further assays were performed on the heart.

GSHPx measurement. The activity of GSHPx (EC 1.11.1.9) in plasma, aortic cytosol and hepatic cytosol was used as an indication of selenium status (Deagen et al., 1987). GSHPx catalyzes the detoxification of lipid peroxides (ROOH) and hydrogen peroxide by the oxidation of reduced glutathione according to the following reaction:

$$2\ GSH + ROOH \xrightarrow[GSHPx]{} GSSG + ROH + H_2O$$

GSHPx activity was assayed by a coupled enzyme procedure using hydrogen peroxide as a substrate. In this assay, GSH is maintained at a constant concentration by exogenous glutathione reductase (GSSG-R) and NADPH which immediately convert any glutathione (GSSG) produced to the reduced form (GSH). The rate of GSSG formation is measured by following the decrease in the absorbance of the reaction mixture at 340 nm as NADPH is converted to NADP.

$$H_2O + GSSG \xrightarrow[NADPH \rightarrow NADP]{GSSG\text{-}R} 2\ GSH$$

At the time of the analysis of the aorta, 0.05 g was minced and homogenized in 2 ml ice-cold 0.1 M phosphate buffer, pH 7.0, containing 0.25M sucrose. The homogenate

was centrifuged at 14,000 x g; the supernatant was used in the reaction. It was centrifuged in a Sorvall RC2B with an SM24 rotor (Dupont Co. Clarement, California). One g of the rat liver was homogenized at the ratio of 1:5 in the buffer described above. The liver homogenates were also centrifuged at 14,000 x g and the supernatant used in the assay. For each liver and plasma GSHPx assay, 0.8 ml reaction mixture, which contained 0.125 M phosphate buffer, pH 7, with 4.5 mM EDTA, 4.7 mM sodium azide, 2.8 nmoles NADPH, 49.9 nmoles reduced glutathione and 0.67 units glutathione reductase, were used. To run the assay 0.1 ml of sample (liver supernatant or plasma) was added to the cuvette, followed by the freshly prepared reaction mixture. Occasionally these samples were too concentrated and were diluted using the phosphate buffer. Because the amount of aorta used was small and the homogenate dilute, 0.2 ml of supernatant was used, rather than the smaller volume as was used with the liver and plasma: this was added to 0.7 ml of reaction mixture.

The reaction was initiated by the addition of 0.1 ml of freshly prepared 0.25 mM H_2O_2 solution. GSHPx activities were measured spectrophotometrically at 340 nm using an Hitashi 100-80A spectrophotometer. The blank cuvette contained double distilled water instead of sample. For each sample and blank, the rate of NADPH oxidation was measured over a period of either 1.5 min or 3 min. The value of the blanks was substracted from those of the

samples before enzyme units were calculated. GSHPx activity is expressed as nmoles NADPH oxidized/min per mg protein. Tissue protein concentration was determined by the method of Lowry et al. (1951).

Selenium concentration. Selenium concentration was determined by a semiautomated fluorometric method (Brown and Watkinson, 1977) using an autoanalyzer II (AlphchemCorp., Clackamas, Oregon) (Beilstein and Whanger, 1986). Samples (plasma, liver, different volumes of 0.303 ppm sodium selenite solution as standards and diets) were digested with nitric acid and perchloric acid, converting selenium to the +4 oxidation state. The pH was adjusted to 2-3 and selenium was complexed with 2,3-diaminonaphthalene (DAN) in a water bath to form 4,5-benzopiazselenol. After extraction with cyclohexane, its fluorescence was measured with a 325nm filter for excitation and a 556 nm filter for emission. Peak heights were measure in mm, a standard curve was plotted and selenium content of samples was calculated from the standard curve in ug/g wet liver weight, ug/ml of plasma or ug/g of diet.

Lipid peroxides. The thiobarbituric acid (TBA) test is one of the most frequently used tests for measuring the peroxidation of fatty acids, tissues and food products. In this test one of the products of peroxidation, malondialdehyde (MDA), is measured. The quantity of this material present is used as an indication of the amount of

peroxidation that has occurred. In this study it was used to analyze the peroxidation in plasma, the aorta and liver.

The first step in the analysis of plasma is to precipitate the protein with trichloracetic acid (TCA). The precipitate is used for further reaction (Satoh, 1978). The initial steps in the analysis of the aorta are to thaw it at room temperature, weigh it, mince it with scissors on ice, and homogenize it in 1.1 ml ice cold pH 7.4 phosphate buffer (0.05 M). For the liver samples one g is thawed and homogenized with 1.15% KCl to make a 10% homogenate. The distillate from the diets (described in the diet section), the precipitated plasma protein, and the homogenate of liver and the aorta were heated with TBA in an acidic solution to produce a red-colored pigment. The reaction that occurs is given in Figure 4. The product absorbs light at 532 nm, fluoresces at 553 nm, and it is extractable into organic solvents such as butanol. MDA in the liver and the plasma was detected spectrometrically and that in the aorta was detected fluorometrically. Because MDA is unstable, the standard is prepared immediately before use by hydrolyzing 1,1,3,3-tetraethoxypropane. The results were expressed in terms of the amounts of MDA produced per g of tissue or ml of plasma. Though a red chromophore is produced in each of the tissues analyzed, each tissue required a different method to prepare the samples. This was necessary to improve sensitivity and to remove possible interferences. In the Satoh method, sialic acid which is present in plasma

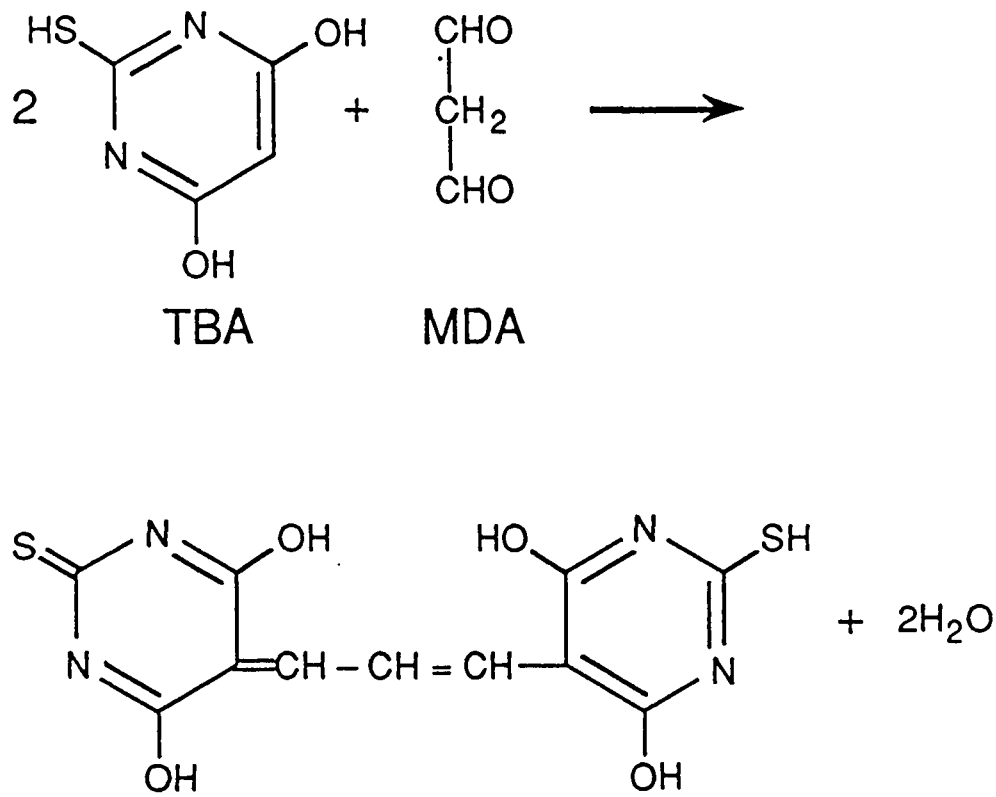

Figure 4. The reaction of TBA test

in abundance and interferes wiht the TBA test, was removed by precipitating protein and lipid peroxides. Detection of MDA fluorometrically allowed us to assay the very small amounts present in the samples of aorta (Suematsu and Abe, 1982). The method which was used to measure liver MDA is the most commonly used (Ohkawa et al., 1979).

Fatty acid profile. The lipids of the liver, plasma, and aorta were extracted with chloroform methanol (1:2 v/v) according to the Bligh and Dyer method (1959). The phospholipids in the liver and aorta were separated by thin layer chromatography using diethyl ether/hexane/acetic acid (30:70:1, v/v/v). The phospholipid fraction, which remained at the origin, was removed from the plate by scraping, eluted with chloroform, and methylated using 14% boron trichloride in methanol (Sigma Chemical Co., St. Louis, Missouri). The fatty acid methyl esters were identified by gas liquid chromatography (Morrison and Smith, 1964) using a Hewlett Packard 5890 Gas Chromatography (GC) interfaced with a Hewlett Packard Chem Work Station. The GC was equipped with a 30 m x 0.25 mm i.d., 0.25 micron film thickness SP 2330 column. Helium was the carrier gas and was used at a flow rate of 1 ml/min with a split ratio of 100:1. Hydrogen and air flow rates were 30 ml per min and 300 ml per min, respectively. Both the injector and detector were maintained at 235^{o}C. The column was programmed for 4 min at 170^{o}C and then a 3^{o}C per min to a maximum temperature of 225^{o}C. Fatty acid methyl esters in the samples were

identified by comparing to authentic standard mixtures (Sigma Chemical Co., St. Louis, Missouri; NuCheck Prep. Inc., Elysian, Minnesota; Supelco Inc. Bellefonte, Pennsylvania).

Radioimmunoassay. The principle of a RIA is competetive antibody binding, where a radioactive antigen competes with a non-radioactive antigen for a fixed number of antibody binding sites. When unlabeled antigen from standards or samples and a fixed amount of tracer (labeled antigen) are allowed to react with a constant and limiting amount of antibody, decreasing amounts of tracer are bound to the antibody as the amount of unlabeled antigen is increased. Dextran-coated charcoal (DCC) was used to separate the antibody-antigen complex from free antigen. The free antigen was adsorbed to DCC and the complex of DCC-free antigen was removed by centrifugation. The supernatant containing the antibody-antigen complex was counted after addition of liquid scintillation cocktail.

TXB_2 was measured in aliquots of suspensions of PPP obtained from platelets aggregated by collagen (Hwang and Carroll, 1980). The PPP samples were used without extracting them and without dilution. 6-keto-PGF_1alpha was measured in aliquots of incubation medium from the aorta (Mitchell et al., 1988). Two kinds of buffers were prepared for the dilution of the stock tracers, the standard solutions, the antibodies, normal rabbit plasma and the RIA reaction medium. One was a phosphate buffered saline

containing gelatin (PBS-gel); the other was phosphate buffered saline containing 0.05 M EDTA (PBS-EDTA). Tracers for the two prostaglandins were from New England Nuclear Products and purchased from Dupont Biotechnology Systems (Wilmington, Delaware). Standards for TXB_2 and 6-keto-PGF_1alpha were purchased from Biomol Research Laboratories Inc. (Plymouth Meeting, Pennsylvania). Antibodies to TXB_2 and 6-keto-PGF_1alpha were generously provided by Dr. D. Hwang (Louisiana State University) and Dr. M. Mathias (Florida State University), respectively. TXB_2 or 6-keto-PGF_1alpha antibodies were diluted at the rate of 1:400. They were then further diluted with normal rabbit plasma (1:400 in PBS-EDTA) to give the final 1:2,000 and 1:12,000 dilution, respectively. Each tube contained either sample or varied amounts of standard substance in a total volume of 0.5 ml. To this was added aliquots of the antibodies (0.2 ml) and the appropriately diluted tracer (approximately 6500 count per min of TXB_2 and 6-keto-PGF_1alpha) in 0.1 ml of PBS-gel buffer, so that the total reaction volume was 0.8 ml. After incubating for 24h at $4^{o}C$, the antibody bound material was separated by adding 0.6 ml of DCC suspension (0.6 mg/ml charcoal, 0.1 mg/ml dextran in distilled water). The tubes were centrifuged for 10 min at 2,300 x g at $4^{o}C$ using a JS7.5 swinging bucket rotor in a J-21C centriguge (Beckman Instrument Inc., Palo Alto, California). The supernatant was decanted into the scintillation vial. To that, 10 ml of liquid scintillation cocktail (Ready

Protein^{+}, Beckman Instrument Inc., Fullerton, California) was added and the radioactivity was determined in a LS 5000 TD Liquid Scintillation System (Beckman Instrument Inc., Fullerton, California).

Platelet aggregation. The PPP and adjusted PRP were used to measure platelet aggregation. ADP (5uM) or collagen (30 ug/ml PRP) were used as agonists. Aggregation of the platelets was measured photometrically using a Chrono-Log aggregometer (Havertown, PA) in siliconized tubes at 37^{o}C. The degree of aggregation in response to an aggregating agent was determined from the maximal increase in light transmission after the introduction of the agonist. Approximately 10 and 90% light transmittance were initially set using unstimulated PRP and PPP respectively, as is shown in Figure 5. In this figure the maximum light transmittance (approximately 80 units) is converted to 100%. The unit measured as maximum platelet aggregation is also converted to percent. Five minutes after the addition of collagen or ADP, the reaction was stopped. The collagen-treated PRP samples were immediately centrifuged at 4^{o}C. After centrifugation, the supernatant was collected for subsequent analysis of TXB_2 by radioimmunoassay (Hwang and Carroll, 1980).

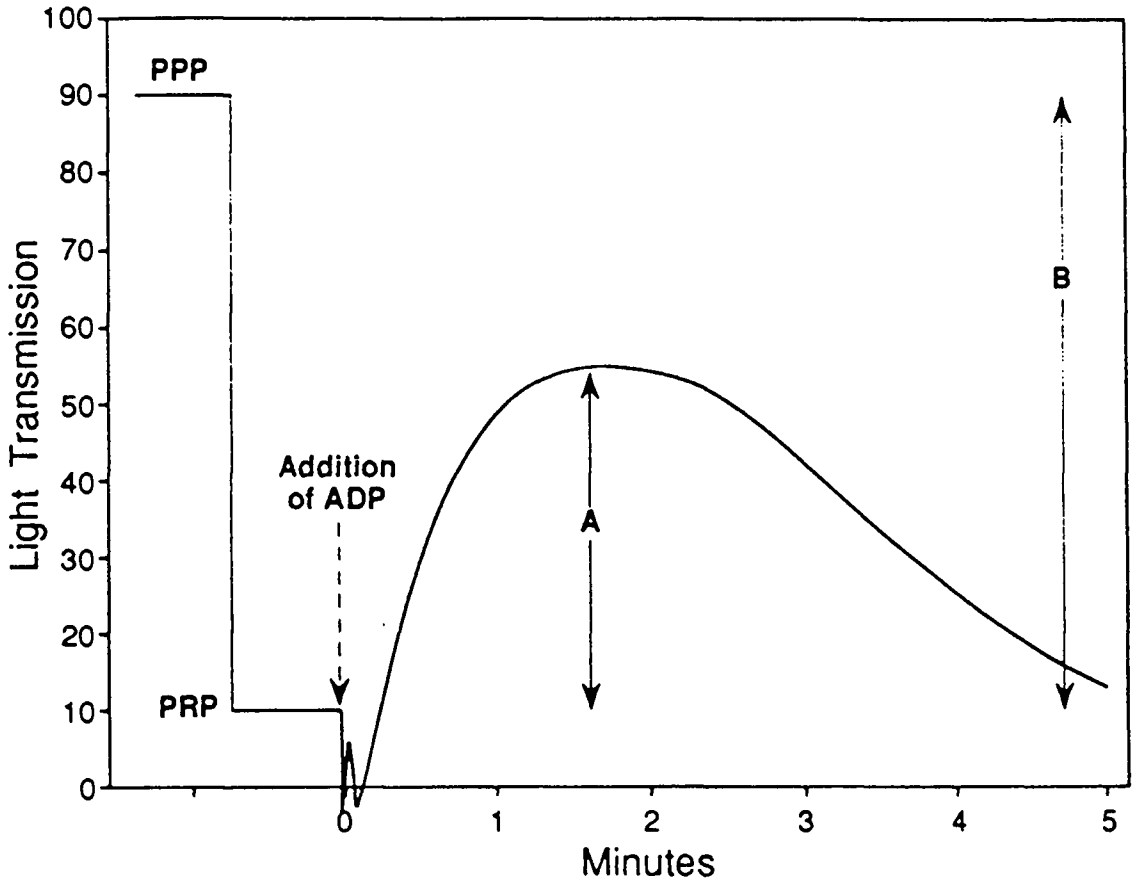

Figure 5. Variables used to measure platelet aggregation

A: maximum aggregation
B: maximum light transmittance
PPP: platelet poor plasma
PRP: platelet rich plasma

Statistical Analysis

Data were assessed by a two way analysis of variance (ANOVA) in a design of two dietary selenium levels and two types of dietary fat using STATGRAPHICS (ver. 3, STSC Inc., Rockville, Maryland) (Snedecor and Cochran, 1980). Since a particular interest of the study was interactive effects between selenium and fish oils, this statistical analysis is especially appropriate. If a significant difference was found for treatment effects, they were partitioned into effects due to the level of dietary selenium, the type of dietary fat and the interaction of these two factors. When necessary to obtain homogeneity of variance, data (such as selenium content in plasma and liver; GSHPx activity in plasma, aorta, and liver; and some of the fatty acids) were transformed into natural logs before statistical analysis. The differences between two treatment groups were compared by the Student's t-test at $P < 0.05$. The relationship between 6-keto-PGF_1alpha production by the aorta, and TXB_2 production by PRP, and collagen- or ADP-stimulated platelet aggregation was evaluated by simple regression analysis (Snedecor and Cochran, 1980).

RESULTS

Body Weight and Tissue Weight

All animals had satisfactory weight gain over the 8 week treatment period. As shown in Table 4, there were no significant differences in food intake and the final weight among the dietary groups. Nor were any differences in heart weight observed. There was, however, a significant effect produced by the type of fat fed on the weight of the liver and the relative liver size (RLS). The feeding of fish oil increased liver weight by 12% (11.2 ± 0.5 vs 12.5 ± 0.3, $P < 0.02$) and the relative liver size by 7% (2.96 ± 0.06 vs 3.18 ± 0.04, $P < 0.002$).

Selenium Status

As shown in Table 5, although there was no significant effect produced by the type of fat fed, selenium supplementation increased selenium content in plasma and liver and increased glutathione peroxidase (GSHPx) activity in plasma, liver, and aorta ($P < 0.001$). The liver was affected more than plasma or the aorta. The increase in the selenium content of the plasma was 13-fold compared to 34-fold in the liver. The increase in GSHPx activity was 19-fold, 12-fold, and 124-fold in the plasma, aorta, and liver respectively. The effect of type of dietary fat on indicators of selenium status was not significant. However, in the selenium-supplemented groups, all indicators of

Table 4

Effect of level of dietary selenium and type of dietary fat on food intake, body weight, heart weight, liver weight and relative liver size[1]

Diets[3]	Food intake	Body weight	Heart	Liver weight	RLS[4]
	g/day	g	g	g	%
-SeCO	18.8±0.6 (n=14)[2]	374.7±13.8 (n=14)	1.18±0.06 (n=13)	11.5±0.7 (n=14)	3.03±0.09
-SeFO	19.2±0.4 (n=13)	393.0± 9.9 (n=13)	1.19±0.05 (n=12)	12.7±0.2 (n=13)	3.23±0.04
+SeCO	18.8±0.5 (n=14)	374.9±13.0 (n=14)	1.12±0.04 (n=12)	10.9±0.6 (n=13)	2.88±0.07
+SeFO	20.0±0.4 (n=13)	395.4±12.6 (n=13)	1.19±0.02 (n=13)	12.4±0.5 (n=13)	3.12±0.06
P-values[5]					
Se	NS	NS	NS	NS	NS
Oil	NS	NS	NS	0.02	0.002
SexOil	NS	NS	NS	NS	NS

[1]Values are means ± SEM.
[2]The number after n= in parenthesis is the number of rats per group.
[3]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.
[4]RLS = relative liver size = weight of the liver (g) divided by the weight of the animal (g) times 100.
[5]NS = Not Significant at $P < 0.05$.

Table 5

Effect of level of dietary selenium and type of dietary fat on tissue selenium concentration and glutathione peroxidase (GSHpx) activity[1]

Diets[3]	Plasma Se (ng Se/ml)	Plasma GSHpx[4]	Aorta GSHpx[4]	Liver Se (ng Se/g tissue) (wet wt)	Liver GSHpx[4]
-SeCO	41.14± 4.27 (n=7)[2]	4.95±0.47 (n=7)	25.39± 1.18 (n=7)	32.63± 2.94 (n=7)	8.00±0.86 (n=7)
-SeFO	43.90± 3.31 (n=7)	4.37±0.67 (n=5)	24.36± 2.2 (n=6)	35.00± 2.75 (n=7)	7.69±0.97 (n=7)
+SeCO	571.59±21.73 (n=7)	91.16±9.43 (n=5)	300.65±14.41 (n=6)	1194.5±39.8 (n=6)	1105.5±48.2 (n=6)
+SeFO	510.75±16.81 (n=6)	84.08±7.69 (n=6)	291.28±18.81 (n=7)	1104.0±29.7 (n=7)	859.0±84.2 (n=7)
P-values[5]					
Se	0.001	0.001	0.001	0.001	0.001
Oil	NS	NS	NS	NS	NS
SexOil	NS	NS	NS	NS	NS

[1]Values are means ± SEM.
[2]The number after n= in parenthesis is the number of rats per group.
[3]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.
[4]Activity is expressed as nanomoles NADPH oxidized per min per mg protein.
[5]NS = Not Significant at $P < 0.05$.

selenium status tended to be lower in the rats that had been fed fish oil. This was especially true of GSHPx activity of liver from rats fed fish oil: the activity was 22% lower in this group. This activity was significant at the $P < 0.05$ level when evaluated using a Student's t-test.

Level of Lipid Peroxides

The effect of the level of dietary selenium and the type of dietary fat on tissue levels of malondialdehyde (MDA) as an indicator of lipid peroxidation is shown in Table 6. Plasma was not affected by the dietary treatments. There was a significant effect from the type of fat used in the diet on the level of MDA in the aorta and liver, however. The concentration of MDA in the aorta of the fish oil group (-SeFO and +SeFO) (68.52 $\pm$ 3.27 nmole/g) was 65% higher than that in corn oil group (-SeCO & +SeCO)(41.52 $\pm$ 6.62 nmole/g, $P < 0.001$). In liver its level (488.57 $\pm$ 23.34) was 17% higher in the fish oil group than in the corn oil group (416.67 $\pm$ 21.16) at $P < 0.02$. In addition, in the liver of rats fed selenium the level of lipid peroxidation was 14% lower than in those not given selenium. This effect was significant at the 6% level of probability rather than the 5% level, however. The level of MDA in the liver of rats fed fish oil without selenium supplementation was higher than that in the liver of rats fed fish oil with selenium supplementation.

Table 6

Effect of level of dietary selenium and type of dietary fat on tissue malondialdehyde level[1]

Diets[3]	Plasma (nmole/ml)	Aorta (nmole/g)	Liver (nmole/g)
-SeCO	1.19±0.14 (n=5)[2]	41.53±1.59 (n=7)	425.51±32.27 (n=7)
-SeFO	1.16±0.16 (n=5)	70.69±6.23 (n=7)	537.10±35.21 (n=7)
+SeCO	1.13±0.09 (n=5)	41.50±2.97 (n=7)	407.83±29.53 (n=7)
+SeFO	1.01±0.05 (n=5)	66.35±2.46 (n=7)	440.04±18.32 (n=7)
P-values[4]			
Se	NS	NS	0.06
Oil	NS	0.001	0.02
SexOil	NS	NS	NS

[1]Values are means ± SEM.
[2]The number after n= in parenthesis is the number of rats per group.
[3]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.
[4]NS = Not Significant at $P < 0.05$.

Fatty Acid Composition

Fatty acid composition of lipids in plasma. The impact of the 8-week treatment on the fatty acid composition of the lipids in plasma is shown in Table 7. An interaction between the level of dietary selenium and the type of dietary fat was significant for two of the fatty acids, 20:0 and 20:3 n-6. Consequently, for these two fatty acids the main effects were not evaluated. The level of 20:0 increased when the corn oil-fed rats were given selenium. When fish oil was fed, the values were so low the difference between the two levels of selenium was negligible. The level of 20:3 n-6 in the plasma dropped when selenium was added to the diet of both oil groups; the drop was larger, however, in the corn oil fed animals.

Because there was no interaction between the level of dietary selenium and the type of dietary fat for most of the fatty acids, the data were reduced (Table 8) to show more clearly the effect of the type of dietary fat on the fatty acid profile. The level of linoleic acid (LA, 18:2 n-6) and arachidonic acid (AA, 20:4 n-6) decreased in the fish oil fed groups. The level of LA from rats fed fish oil was 73% of that from rats fed corn oil. The level of AA from rats fed fish oil was 30% of that from rats fed corn oil.

In contrast, the levels of eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) in rats fed fish oil were 62 times and 7 times higher respectively than in rats fed corn oil. The amount of AA decreased by

Table 7

Effect of level of dietary selenium and type of dietary fat on the fatty acid composition of plasma total lipids[1,2]

Fatty acid	Diets[3] -SeCO	-SeFO	+SeCO	+SeFO	P-values[4] Se	Oil	SexOil
14:0	0.28±0.03	0.44±0.05	0.26±0.04	0.48±0.07	NS	0.002	NS
15:0	0.24±0.02	0.36±0.03	0.24±0.01	0.33±0.03	NS	0.001	NS
16:0	18.86±0.62	20.97±0.88	18.24±0.58	21.48±0.61	NS	0.001	NS
16:1n-7	1.24±0.18	2.39±0.25	0.81±0.24	2.31±0.13	NS	0.001	NS
17:0	0.43±0.03	0.64±0.05	0.60±0.13	0.59±0.06	NS	NS	NS
18:0	9.64±0.29	9.76±0.59	10.93±1.07	10.56±0.34	NS	NS	NS
18:1n-9	8.83±0.54	9.93±0.88	7.32±0.70	10.10±0.82	NS	0.02	NS
18:1n-7	2.64±0.20	2.50±0.05	2.28±0.19	2.51±0.19	NS	NS	NS
18:2n-6	22.25±0.63	16.14±0.59	20.44±1.46	14.97±0.62	NS	0.001	NS
18:3n-6	0.47±0.07	0.05±0.02	0.28±0.04	0.03±0.02	0.02	0.001	NS
20:0	0.05±0.03	0.02±0.02	0.12±0.01	0	NS	0.001	0.05
18:3n-3	0.25±0.01	0.28±0.02	0.20±0.03	0.28±0.02	NS	0.01	NS
20:1n-9	0.07±0.02	0.04±0.02	0.09±0.05	0.02±0.02	NS	NS	NS
18:4n-3	0	0.14±0.01	0	0.17±0.01	NS	0.001	NS
21:0	0	0.14±0.04	0	0.18±0.01	NS	0.01	NS
20:2n-6	0.34±0.05	0.11±0.06	0.25±0.01	0	0.03	0.001	NS
20:3n-6	0.60±0.06	0.74±0.02	0.30±0.02	0.67±0.01	0.001	0.001	0.004
22:0	0.25±0.02	0.22±0.02	0.24±0.02	0.19±0.02	NS	NS	NS
20:4n-6	27.84±1.5	10.86±0.72	32.26±1.77	11.19±0.51	NS	0.001	NS
22:1n-9	0	0	0	0	NS	NS	NS
20:5n-3	0.14±0.01	9.21±0.75	0.13±0.04	8.37±0.57	NS	0.001	NS
24:0	0.83±0.1	0.63±0.05	0.79±0.15	0.62±0.09	NS	NS	NS
22:4n-6	0.62±0.07	0	0.56±0.09	0	NS	0.001	NS
24:1n-9	0.69±0.08	1.14±0.09	0.51+0.03	1.04±0.06	NS	0.001	NS
22:5n-6	0.64±0.13	0.12±0.03	0.55±0.07	0.11±0.03	NS	0.001	NS
22:4n-3	1.36±0.22	0.78±0.28	0.49±0.13	0.36±0.05	0.004	NS	NS
22:5n-3	0.28±0.02	2.01±0.17	0.30±0.02	2.06±0.11	NS	0.001	NS
22:6n-3[5]	1.28±0.12	10.36±0.57	1.70±0.22	11.39±0.44	0.04	0.001	NS
total PUFA[6]	56.06±1.30	50.80±1.37	57.45±0.84	49.62±0.74	NS	0.001	NS
total n-6	52.75±1.20	28.03±1.20	54.64±0.95	26.98±0.61	NS	0.001	NS
total n-3	3.31±0.12	22.77±0.41	2.81±0.17	22.64±0.80	NS	0.001	NS
n-3/n-6	0.06±0.00	0.82±0.03	0.05±0.00	0.84±0.04	NS	0.001	NS
(epa/aa)*10[7]	0.05±0.01	8.47±0.31	0.04±0.01	7.49±0.42	NS	0.001	NS

[1]Expressed as the weight percent of the fatty acid methyl esters (FAMEs).
[2]Values are means ± SEM, n= 5 rats per group.
[3]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.
[4]NS = Not significant at $P < 0.05$.
[5]The data for this fatty acid was subjected to the transformation to natural log to obtain homogeneity of variance.
[6]Total PUFA is the summation of the weight percent of each fatty acid which contains 2 or more than 2 double bonds.
[7](epa/aa)*10 denotes 10 times the ratio of the weight percent of eicosapentaenoic acid to that of arachidonic acid.

Table 8

Effect of type of dietary fat on the fatty acid composition of plasma total lipids[1,2]

Fatty acid	Diets[3] Corn oil (-SeCO & +SeCO)	Fish oil (-SeFO & +SeFO)	P-values[4]
14:0	0.27 ± 0.02	0.46 ± 0.04	0.002
15:0	0.24 ± 0.01	0.34 ± 0.02	0.001
16:0	18.55 ± 0.41	21.22 ± 0.51	0.001
16:1n-7	1.02 ± 0.16	2.35 ± 0.14	0.001
17:0	0.52 ± 0.07	0.62 ± 0.04	NS
18:0	10.29 ± 0.56	10.16 ± 0.35	NS
18:1n-9	8.08 ± 0.49	10.02 ± 0.57	0.02
18:1n-7	2.46 ± 0.14	2.50 ± 0.09	NS
18:2n-6	21.35 ± 0.81	15.56 ± 0.45	0.001
18:3n-6	0.38 ± 0.04	0.04 ± 0.01	0.001
20:0	0.08 ± 0.02	0	0.001
18:3n-3	0.23 ± 0.02	0.28 ± 0.01	0.01
20:1n-9	0.08 ± 0.03	0.03 ± 0.01	NS
18:4n-3	0	0.15 ± 0.01	0.001
21:0	0	0.16 ± 0.02	0.01
20:2n-6	0.30 ± 0.03	0.06 ± 0.03	0.001
22:0	0.25 ± 0.01	0.21 ± 0.02	NS
20:4n-6	30.05 ± 1.32	11.03 ± 0.42	0.001
22:1n-9	0	0	NS
20:5n-3	0.14 ± 0.02	8.79 ± 0.47	0.001
24:0	0.81 ± 0.08	0.63 ± 0.05	NS
22:4n-6	0.59 ± 0.06	0	0.001
24:1n-9	0.60 ± 0.05	1.09 ±0.059	0.001
22:5n-6	0.59 ± 0.07	0.12 ±0.023	0.001
22:4n-3	0.92 ± 0.19	0.57 ± 0.15	NS
22:5n-3	0.29 ± 0.01	2.04 ± 0.09	0.001
22:6n-3	1.49 ± 0.14	10.87 ± 0.38	0.001
total PUFA[5]	56.76 ± 0.77	50.21 ± 0.76	0.001
total n-6	53.70 ± 0.79	27.51 ± 0.66	0.001
total n-3	3.06 ± 0.13	22.77 ± 0.42	0.001
n-3/n-6	0.06 ± 0.00	0.83 ± 0.03	0.001
(epa/aa)*10[6]	0.05 ± 0.01	7.98 ± 0.29	0.001

[1]Expressed as the weight percent of the fatty acid methyl esters.
[2]Values are means ± SEM, n= 10 rats per group.
[3]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.
[4]NS = Not significant at $P < 0.05$.
[5]Total PUFA is the summation of the weight percent of each fatty acid which contains 2 or more than 2 double bonds.
[6](epa/aa)*10 denotes 10 times the ratio of the weight percent of eicosapentaenoic acid to that of arachidonic acid.

the feeding of fish oil seemed to be replaced approximately half by EPA and half by DHA. The level of all fatty acids of the n-6 family (18:2 n-6, 18:3 n-6, 20:2 n-6, 20:4 n-6, 22:4 n-6 and 22:5n-6) except for 20:3 n-6 was decreased in the plasma of fish oil-fed rats. The levels of the fatty acids in the n-3 family (18:3 n-3, 18:4 n-3, 20:5 n-3, 22:5 n-3 and 22:6 n-3) were increased. This resulted in the ratio of n-3 to n-6 fatty acids and EPA to AA levels increasing. The levels of the saturated fatty acids, 14:0 and 16:0, increased. The levels of the odd-chained fatty acids, 15:0 and 21:0, and the three monounsaturated fatty acids, 16:1 n-7, 18:1 n-9 and 24:1 n-9 also increased. Overall, the weight percent of total PUFA decreased.

The changes in the levels of the fatty acids from selenium deficiency are shown in Table 9. Only fatty acids in which there was a significant effect are given. In the animals given the low selenium diet, the level of the n-6 fatty acids, 18:3 n-6 and 20:2 n-6 increased significantly ($P < 0.02$ and $P < 0.03$). A slight decrease, significant only at $P < 0.07$, could be observed in 20:4 n-6. As AA comprised a greater percent of the total lipids than any other n-6 fatty acid, a small change in AA levels might be important. From the n-3 family, the level of 22:4 n-3 was increased ($P < 0.004$) and 22:6 n-3 was decreased ($P < 0.04$) in the low selenium group.

Fatty acid composition of phospholipids from the aorta. When fish oil was included in the diet except for 22:6 n-3,

Table 9

Effect of level of dietary selenium on the fatty acid composition in plasma, aorta and liver[1,2,3]

Fatty acid	Diets[4] Low selenium (-SeCO & -SeFO)	High selenium (+SeCO & +SeFO)	P-values
plasma			
18:3 n-6	0.26 ± 0.08	0.15 ± 0.05	0.02
20:2 n-6	0.23 ± 0.05	0.13 ± 0.04	0.03
20:4 n-6	19.35 ± 2.94	21.73 ± 3.62	0.07
22:4 n-3	1.07 ± 0.19	0.43 ± 0.07	0.004
22:6 n-3[5]	5.82 ± 1.54	6.54 ± 1.63	0.04
aorta			
22:5 n-3	2.34 ± 0.44	2.53 ± 0.46	0.02
22:6 n-3	3.47 ± 0.59	3.90 ± 0.71	0.05
n-3/n-6	0.41 ± 0.09	0.45 ± 0.10	0.01
liver			
24:1 n-9	0.32 ± 0.09	0.36 ± 0.10	0.05

[1]Expressed as the weight percent of the fatty acid methyl esters.
[2]Values are means ± SEM, n = 10 rats per group for plasma; n = 13 rats per group for aorta and liver.
[3]These fatty acids are those influenced significantly by selenium supplementation and are excerpted from Tables 7, 10 and 12.
[4]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.
[5]The data for this fatty acid were subjected to the transformation to natural log to obtain homogeneity of variance.

there was no interaction with selenium level in the composition of the fatty acids in the phospholipids from the aorta (Table 10). The effect of the lack of selenium in the diet on 22:6 n-3 varied according to the type of dietary fat. In the corn oil fed animals the lack of selenium in the diets caused a small increase in the level of DHA, whereas it caused a statistically significant decrease in DHA level in the fish oil fed animals. This interaction implied that there was a minor effect from the type of dietary fat at low and high levels of selenium.

The data are reduced in Table 11 to show the effect of the type of dietary fat on the fatty acid profile of phospholipids from the aorta. In contrast to observations in the plasma and liver, the 18:2 n-6 content of aortic phospholipids was increased in the fish oil fed groups ($P < 0.02$). There was the expected reduction in the arachidonic acid levels. AA in phospholipids of the fish oil fed rats was 55% of that in the corn oil fed rats ($P < 0.001$). The level of 20:5 n-3 in phospholipids was 17 times greater in the fish oil fed group than in the corn oil fed group ($P < 0.001$). The level of 22:6 n-3 in aortic phospholipids was 4 times greater in the fish oil fed group than in the corn oil fed group. Again the level of the fatty acids in the n-6 family (20:2 n-6, 20:4 n-6, 22:4 n-6 and 22:5 n-6) except for 20:3 n-6 was decreased. The level of 22:4 n-6 in aortic phospholipids in the corn oil fed group was large compared

Table 10

Effect of level of dietary selenium and type of dietary fat on the fatty acid composition of phospholipids from the aorta[1,2]

Fatty acid	Diets[3] -SeCO (n=6)	-SeFO (n=7)	+SeCO (n=6)	+SeFO (n=7)	P-values[4] Se	Oil	SexOil
14:0	0.73±0.10	0.88±0.05	0.82±0.13	0.82±0.05	NS	NS	NS
15:0	0.55±0.05	0.63±0.07	0.60±0.07	0.54±0.06	NS	NS	NS
16:0	19.67±0.75	20.99±0.32	20.66±0.56	20.47±0.22	NS	NS	NS
16:1n-7	0.55±0.02	1.15±0.05	0.63±0.03	1.15±0.06	NS	0.001	NS
17:0	1.16±0.11	1.02±0.10	1.12±0.12	0.80±0.09	NS	0.04	NS
18:0	24.88±1.19	23.23±0.46	24.43±0.68	21.65±0.15	NS	0.004	NS
18:1n-9	6.48±0.17	7.83±0.11	6.46±0.16	8.21±0.24	NS	0.001	NS
18:1n-7	3.89±0.21	4.07±0.06	4.05±0.11	4.29±0.08	NS	NS	NS
19:0	0.09±0.03	0.05±0.02	0.04±0.03	0.12±0.03	NS	NS	NS
18:2n-6	5.77±0.62	6.71±0.38	5.05±0.37	6.26±0.26	NS	0.02	NS
18:3n-6	0	0	0	0.03±0.03	NS	NS	NS
20:0	0.76±0.05	0.74±0.05	1.30±0.30	0.80±0.05	NS	NS	NS
18:3n-3	0	0.04±0.02	0	0.07±0.05	NS	NS	NS
21:0	0.24±0.15	0.11±0.03	0.36±0.21	0.10±0.03	NS	NS	NS
20:2n-6	0.67±0.12	0.24±0.02	0.57±0.06	0.25±0.02	NS	0.001	NS
20:3n-6	1.06±0.06	1.47±0.04	0.96±0.08	1.46±0.05	NS	0.001	NS
22:0	1.10±0.07	1.20±0.06	1.18±0.05	1.26±0.07	NS	NS	NS
20:4n-6	18.39±1.41	9.79±0.21	18.23±0.95	10.44±0.18	NS	0.001	NS
22:1n-9	1.39±0.11	0.93±0.27	1.08±0.34	0.81±0.12	NS	NS	NS
20:5n-3	0.20±0.04	3.89±0.16	0.25±0.03	3.99±0.12	NS	0.001	NS
24:0	2.03±0.19	2.58±0.05	2.15±0.08	2.83±0.11	NS	0.001	NS
22:4n-6	6.27±0.18	0	6.02±0.23	0	NS	0.001	NS
24:1n-9	0	2.49±0.1	0	2.60±0.07	NS	0.001	NS
22:5n-6	1.60±0.17	0.48±0.05	1.67±0.1	0.34±0.04	NS	0.001	NS
22:4n-3	0.06±0.06	0.12±0.02	0.14±0.08	0.12±0.01	NS	NS	NS
22:5n-3	0.70±0.04	3.74±0.05	0.82±0.05	4.00±0.12	0.02	0.001	NS
22:6n-3	1.34±0.10	5.29±0.28	1.27±0.14	6.14±0.20	0.05	0.001	0.03
total PUFA[5]	36.07±2.08	31.83±0.43	34.97±1.20	33.11±0.38	NS	0.01	NS
total n-6	33.77±2.10	18.70±0.24	32.50±1.13	18.78±0.21	NS	0.001	NS
total n-3	2.30±0.11	13.88±0.95	2.47±0.10	14.32±0.32	NS	0.001	NS
n-3/n-6	0.07±0.01	0.70±0.02	0.08±0.00	0.76±0.02	0.01	0.001	NS
(epa/aa)*10[6]	0.12±0.03	3.99±0.20	0.13±0.01	3.82±0.11	NS	0.001	NS

[1]Expressed as the weight percent of the fatty acid methyl esters.
[2]Values are means ± SEM. The number after n= in the parenthesis is the number of rats per group.
[3]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.
[4]NS = Not significant at $P < 0.05$.
[5]Total PUFA is the summation of the weight percent of each fatty acid which contains 2 or more than 2 double bonds.
[6](epa/aa)*10 denotes 10 times the ratio of the weight percent of eicosapentaenoic acid to that of arachidonic acid.

Table 11

Effect of type of dietary fat on the fatty acid composition of phospholipids from the aorta[1,2]

Fatty acid	Diets[3] Corn oil (n=12) (-SeCO & +SeCO)	Fish oil (n=14) (-SeFO & +SeFO)	P-values[4]
14:0	0.78 ± 0.08	0.85 ± 0.04	NS
15:0	0.57 ± 0.04	0.59 ± 0.05	NS
16:0	20.17 ± 0.47	20.73 ± 0.20	NS
16:1n-7	0.59 ± 0.02	1.15 ± 0.04	0.001
17:0	1.14 ± 0.08	0.91 ± 0.07	0.04
18:0	24.65 ± 0.66	22.45 ± 0.32	0.004
18:1n-9	6.47 ± 0.11	8.02 ± 0.14	0.001
18:1n-7	3.97 ± 0.11	4.18 ± 0.06	NS
19:0	0.07 ± 0.02	0.09 ± 0.02	NS
18:2n-6	5.41 ± 0.36	6.49 ± 0.23	0.02
18:3n-6	0	0.16 ± 0.16	NS
20:0	1.03 ± 0.17	0.77 ± 0.04	NS
18:3n-3	0	0.06 ± 0.02	0.06
21:0	0.30 ± 0.13	0.10 ± 0.02	NS
20:2n-6	0.62 ±0.06	0.25 ± 0.01	0.001
20:3n-6	1.01 ± 0.05	1.46 ± 0.03	0.001
22:0	1.14 ± 0.04	1.23 ± 0.04	NS
20:4n-6	18.31 ± 0.81	10.12 ± 0.16	0.001
22:1n-9	1.23 ± 0.18	0.87 ± 0.14	NS
20:5n-3	0.22 ± 0.02	3.94 ± 0.10	0.001
24:0	2.09 ± 0.10	2.70 ± 0.07	0.001
22:4n-6	6.15 ± 0.14	0	0.001
24:1n-9	0	2.54 ± 0.06	0.001
22:5n-6	1.64 ± 0.09	0.41 ± 0.03	0.001
22:4n-3	0.10 ± 0.05	0.12 ± 0.01	NS
22:5n-3	0.76 ± 0.04	3.87 ± 0.07	0.001
22:6n-3	1.31 ± 0.08	5.72 ± 0.20	0.001
total PUFA[5]	35.52 ± 1.16	31.47 ± 0.33	0.01
total n-6	33.13 ± 1.15	18.74 ± 0.15	0.001
total n-3	2.39 ± 0.08	14.10 ± 0.49	0.001
n-3/n-6	0.07 ± 0.00	0.73 ± 0.02	0.001
(epa/aa)*10[6]	0.12 ± 0.02	3.91 ± 0.11	0.001

[1]Expressed as the weight percent of the fatty acid methyl esters.
[2]Values are means ± SEM. The number after n= in parenthesis is the number of rats per group.
[3]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.
[4]NS = Not Significant at $P < 0.05$.
[5]Total PUFA is the summation of the weight percent of each fatty acid which contains 2 or more than 2 double bonds.
[6](epa/aa)*10 denotes 10 times the ratio of the weight percent of eicosapentaenoic acid to that of arachidonic acid.

to that in other tissues. While the level of 20:4 n-6 in phospholipids in aorta from rats fed corn oil was smaller than that in plasma and liver, the level of 22:4 n-6 in phospholipids from aorta of rats fed corn oil comprised a significantly larger weight percent compared to that in plasma and liver. The level of fatty acids from the n-3 family (20:5 n-3, 22:5 n-3 and 22:6 n-3) was increased ($P < 0.001$). This resulted in the ratio of n-3 to n-6 fatty acids and EPA to AA levels increasing. The levels of the saturated fatty acid, 18:0 decreased, while 24:0 increased. The levels of the odd-chained fatty acid, 17:0, decreased. The three monounsaturated fatty acids, 16:1 n-7, 18:1 n-9, and 24:1 n-9 increased. The weight percent of total PUFA decreased.

In a few of the fatty acids there was a significant effect due to the level of dietary selenium. The reduction of these data is shown in Table 9. The lack of selenium decreased the weight percent of 22:5 n-3 and the ratio n-3 to n-6 fatty acids, although the changes were small ($P < 0.02$ and $P < 0.01$).

Fatty acid composition of phospholipids from the liver. The fatty acid composition of the liver phospholipids after the 8 week feeding period is shown in Table 12. Except for 20:2 n-6, there was no interaction. The effect of selenium supplementation on the level of 20:2 n-6 varied according to the type of fat. Because an interaction was significant but much smaller than the main effect from the type of dietary

Table 12

Effect of level of dietary selenium and type of dietary fat on the fatty acid composition of phospholipids from the liver[1,2]

Fatty acid	Diets[3] -SeCO (n=6)	-SeFO (n=7)	+SeCO (n=6)	+SeFO (n=7)	P-values[4] Se	Oil	SexOil
14:0	0.19±0.03	0.20±0.01	0.21±0.04	0.24±0.03	NS	NS	NS
15:0	0.21±0.01	0.24±0.01	0.19±0.03	0.25±0.03	NS	NS	NS
16:0	17.50±0.38	20.53±0.57	16.73±0.46	20.43±0.39	NS	0.001	NS
16:1n-7	0.56±0.08	1.19±0.06	0.57±0.11	1.09±0.11	NS	0.001	NS
17:0	0.77±0.04	0.82±0.04	0.78±0.08	0.90±0.09	NS	NS	NS
18:0	23.22±0.71	21.80±0.51	23.40±0.58	22.66±0.51	NS	NS	NS
18:1n-9	2.90±0.13	3.78±0.10	3.01±0.17	3.63±0.25	NS	0.001	NS
18:1n-7	3.27±0.34	2.77±0.07	3.19±0.25	2.86±0.16	NS	NS	NS
19:0	0.12±0.03	0.12±0.01	0.13±0.02	0.14±0.01	NS	NS	NS
18:2n-6	12.61±0.49	13.20±0.21	12.66±0.25	12.86±0.50	NS	NS	NS
18:3n-6	0.02±0.02	0.01±0.01	0.05±0.03	0	NS	0.05	NS
20:0	0.18±0.02	0.10±0.02	0.16±0.02	0.14±0.01	NS	0.03	NS
18:3n-3	0	0.06±0.02	0.01+0.01	0.06±0.02	NS	0.001	NS
20:1n-9	0.24±0.03	0.13±0.04	0.17±0.01	0.11±0.04	NS	0.03	NS
18:4n-3	0	0.03±0.03	0	0.01±0.01	NS	NS	NS
21:0	0.02±0.01	0.13±0.02	0	0.16±0.05	NS	0.001	NS
20:2n-6	0.49±0.04	0.15±0.01	0.41±0.03	0.17±0.01	NS	0.001	0.05
20:3n-6	0.70±0.11	1.17±0.06	0.52±0.11	1.15±0.07	NS	0.001	NS
22:0	2.07±0.15	1.40±0.30	1.98±0.20	1.29±0.24	NS	0.01	NS
20:4n-6	29.10±0.34	12.68±0.31	29.66±0.49	12.42±0.26	NS	0.001	NS
22:1n-9	0	0	0	0.13±0.10	NS	NS	NS
20:5n-3	0.06±0.01	4.80±0.32	0.06±0.02	4.67±0.34	NS	0.001	NS
24:0	0.90±0.04	0.67±0.04	0.93±0.05	0.73±0.03	NS	0.001	NS
22:4n-6	0.83+0.02	0	0.81±0.03	0	NS	0.001	NS
24:1n-9	0	0.59±0.01	0	0.67±0.04	0.05	0.001	NS
22:5n-6	0.66±0.10	0.20±0.01	0.60±0.05	0.21±0.01	NS	0.001	NS
22:4n-3	0.07±0.02	0.03±0.01	0.05±0.01	0.05±0.01	NS	NS	NS
22:5n-3	0.58±0.03	2.26±0.08	0.59±0.05	2.10±0.14	NS	0.001	NS
22:6n-3	2.78±0.16	10.94±0.21	3.16±0.18	10.94±0.47	NS	0.001	NS
total PUFA[5]	47.82±0·43	45.39±0·37	48.55±0.44	44.58±0.31	NS	0.001	NS
total n-6	44.40±0·42	27.40±0·42	44.69±0.30	26.80±0.67	NS	0.001	NS
total n-3	3.48±0.18	18.13±0.44	3.87±0.22	17.78±0.86	NS	0.001	NS
n-3/n-6	0.08±0.00	0.66±0.02	0.09±0.00	0.67±0.05	NS	0.001	NS
(epa/aa)*10[6]	0.02±0.00	3.82±0.30	0.02±0.01	3.80±0.35	NS	0.001	NS

[1]Expressed as the weight percent of the fatty acid methyl esters.
[2]Values are means ± SEM. The number after n= in the parenthesis is the number of rats per group.
[3]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.
[4]NS = Not significant at $P < 0.05$.
[5]Total PUFA is the summation of the weight percent of each fatty acid which contains 2 or more than 2 double bonds.
[6](epa/aa)*10 denotes 10 times the ratio of the weight percent of eicosapentaenoic acid to that of arachidonic acid.

fat, interaction implied that there was a minor variation in the effect of type of fat according to the level of dietary selenium. The level of 20:2 n-6 decreased in the corn oil fed animals but increased in the fish oil fed ones. Although these changes were significant, they affected only a small amount of the total fatty acid composition of the liver.

There were significant effects of fish oil feeding on the weight percent of many of the fatty acids. Consequently, the data are reduced according to the type of fat and are shown in Table 13. Level of 18:2 n-6 did not differ significantly between any of the dietary groups. The amount of 20:4 n-6 in hepatic phospholipids from the fish oil fed group was 45% of its amount from the corn oil fed group ($P < 0.001$). The level of 20:5 n-3 was 79 times higher in the fish oil fed group than in the corn oil one ($P < 0.001$). The levels of 22:6 n-3 were only 4 times higher in the fish oil fed group than in corn oil fed group ($P < 0.001$). The amount of DHA was twice that of EPA in the fish oil fed group. Most of the fatty acids in the n-6 family (18:3 n-6, 20:2 n-6, 20:4 n-6, 22:4 n-6 and 22:5 n-6) were decreased, only the level of 20:3 n-6 was increased. In the n-3 family, in addition to the increase in 20:5 n-3 and 22:6 n-3, the levels of 22:5 n-3 and 18:3 n-3 were also increased ($P < 0.001$). This resulted in the ratio of n-3 to n-6 fatty acids and EPA to AA levels increasing. The level of the saturated fatty acid, 16:0, increased while 20:0, 22:0 and

Table 13

Effect of type of dietary fat on fatty acid composition of phospholipids from the liver[1,2]

Fatty acid	Diets[3] Corn oil (n=12) (-SeCO & +SeCO)	Fish oil (n=14) (-SeFO & +SeFO)	P-values[4]
14:0	0.20 ± 0.02	0.22 ± 0.02	NS
15:0	0.20 ± 0.01	0.25 ± 0.02	NS
16:0	17.12 ± 0.31	20.50 ± 0.33	0.001
16:1n-7	0.55 ± 0.07	1.07 ± 0.09	0.001
17:0	0.78 ± 0.04	0.86 ± 0.05	NS
18:0	23.32 ± 0.44	22.25 ± 0.37	NS
18:1n-9	2.96 ± 0.10	3.71 ± 0.13	0.001
18:1n-7	3.23 ± 0.20	2.82 ± 0.09	NS
19:0	0.12 ± 0.02	0.13 ± 0.01	NS
18:2n-6	12.64 ± 0.26	13.04 ± 0.26	NS
18:3n-6	0.03 ± 0.02	0	0.05
20:0	0.17 ± 0.02	0.12 ± 0.01	0.03
18:3n-3	0	0.06 ±0.01	0.001
20:1n-9	0.20 ± 0.02	0.12 ± 0.03	0.03
18:4n-3	0	0.02 ± 0.02	NS
21:0	0.01 ± 0.01	0.14 ± 0.03	0.001
20:2n-6	0.45 ± 0.02	0.16 ± 0.01	0.001
20:3n-6	0.61 ± 0.08	1.16 ± 0.04	0.001
22:0	2.02 ± 0.12	1.34 ± 0.19	0.01
20:4n-6	29.38 ± 0.29	12.56 ± 0.20	0.001
22:1n-9	0	0	NS
20:5n-3	0.06 ± 0.01	4.74 ± 0.23	0.001
24:0	0.92 ± 0.03	0.70 ± 0.03	0.001
22:4n-6	0.82 ± 0.02	0	0.001
24:1n-9	0	0.64 ± 0.02	0.001
22:5n-6	0.63 ± 0.05	0.21 ± 0.01	0.001
22:4n-3	0.06 ± 0.01	0.04 ± 0.00	NS
22:5n-3	0.58 ± 0.03	2.17 ± 0.08	0.001
22:6n-3	2.97 ± 0.13	10.95 ± 0.25	0.001
total PUFA[5]	48.19 ± 0.31	44.99 ± 0.26	0.001
total n-6	44.54 ± 0.25	27.10 ± 0.39	0.001
total n-3	3.67 ± 0.15	18.13 ± 0.47	0.001
n-3/n-6	0.08 ± 0.00	0.66 ± 0.02	0.001
(epa/aa)*10[6]	0.02 ± 0.00	3.81 ± 0.22	0.001

[1]Expressed as the weight percent of the fatty acid methyl esters.
[2]Values are means ± SEM. The number after n= in parenthesis is the number of rats per group.
[3]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.
[4]NS = Not Significant at $P < 0.05$.
[5]Total PUFA is the summation of the weight percent of each fatty acid which contains 2 or more than 2 double bonds.
[6](epa/aa)*10 denotes 10 times the ratio of the weight percent of eicosapentaenoic acid to that of arachidonic acid.

24:0 decreased. The level of the odd-chained fatty acid, 21:0, increased. The levels of the monounsaturated fatty acids, 16:1 n-7, 18:1 n-9, and 24:1 n-9 increased, while 20:1 n-9 decreased. The increase of 18:1 n-9 and 16:0 in fish oil group led to the decrease of weight percent of total PUFA.

The fatty acid composition of the hepatic phospholipids from the selenium-supplemented and selenium-deficient groups was very similar. Selenium supplementation increased the level of 24:1 n-9 as shown in Table 9.

Prostaglandin Synthesis

Thromboxane B_2 synthesis. As shown in Table 14, there was an interaction between the level of dietary selenium and the type of fat fed in the production of TXB_2 in collagen-stimulated platelet rich plasma (PRP)($P < 0.05$). As expected, fish oil feeding resulted in much lower levels of TXB_2 but the difference in TXB_2 synthesis between the corn oil fed rats and fish oil fed rats was greater at the low dietary selenium level than at the adequate selenium level. At the high dietary selenium level, the production of TXB_2 by fish oil fed rats was 36% less than that of corn oil fed rats whereas at the low dietary selenium level the production by fish oil fed rats was 58% less than that of the corn oil fed animals. The interaction was significant but much smaller than the main effect of the type of dietary fat.

Table 14

Effect of level of dietary selenium and type of dietary fat on thromboxane B_2 formation in collagen-stimulated PRP and synthesis of 6-keto PGF_1alpha by incubated segment of aorta[1]

Diets[3]	TXB_2 (ng/ml PRP)	6-keto-PGF_1alpha (ng /mg aorta)
-SeCO	48.2 ± 2.3 (n=7)[2]	13.7 ± 1.2 (n=7)
-SeFO	20.1 ± 2.9 (n=8)	5.6 ± 0.9 (n=7)
+SeCO	40.0 ± 4.8 (n=7)	8.9 ± 1.4 (n=6)
+SeFO	25.6 ± 2.9 (n=7)	6.3 ± 0.8 (n=7)
P-values[4]		
Se	NS	NS
Oil	0.001	0.001
SexOil	0.05	0.02

[1]Values are means ± SEM.
[2]The number after n= in the parenthesis is the number of rats per each group.
[3]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.
[4]NS = Not Significant at $P < 0.05$.

6-keto PGF_1alpha synthesis. Prostacyclin (PGI_2) production in incubated segments of aorta was assessed by the measurement of its stable hydrolysis product, 6-keto-PGF_1alpha. The production of 6-keto-PGF_1alpha for each dietary group is shown in Table 14. Again there was an interaction between the level of dietary selenium and the type of dietary fat. The effect of fish oil feeding on PGI_2 synthesis was not seen at the higher selenium level: the PGI_2 production from rats fed fish oil was very similar to that obtained from the rats fed corn oil. However, rats fed fish oil without selenium supplementation produced 60% less PGI_2 than rats fed the corn oil diets, an amount which was statistically significant. The corn oil feeding with low dietary selenium caused an increase in PGI_2 production compared to corn oil feeding with high dietary selenium.

Hemostatic function

Platelet aggregation. As shown in Table 15, platelet rich plasma (PRP) from rats fed fish oil showed 24% less platelet aggregation than PRP from rats fed corn oil when stimulated by ADP (5uM) (44.07 ± 2.97 vs 34.34 ± 3.16, $P < 0.05$). Though there was no statistically significant interaction between the level of dietary selenium and the type of dietary fat, rats fed fish oil had 33% less ADP-induced platelet aggregation than rats fed corn oil when they were given selenium adequate diets. In contrast, at the low level of dietary selenium, the difference was half

Table 15

Effect of level of dietary selenium and the type of dietary fat on platelet aggregation induced by ADP and collagen[1,2]

Diets[3]	Agonist ADP (5uM)	collagen(30 ug/ml PRP)
-SeCO	44.7±4.9 (n=6)	45.2±7.5 (n=9)
-SeFO	37.7±4.1 (n=9)	39.9±7.6 (n=9)
+SeCO	43.6±4.0 (n=7)	36.5±10.2 (n=7)
+SeFO	29.3±4.7 (n=6)	36.7±7.3 (n=11)
P-values[4]		
Se	NS	NS
Oil	0.03	NS
SexOil	NS	NS

[1]Values are means ± SEM. The number after n= in parenthesis is the number of rats per group.
[2]The degree of aggregation induced by the agonists (either ADP or collagen) was determined from the maximal increase in light transmittance after the introduction of the agonist. Since the maximal light transmittance (approximately 80 units) was converted to 100%, the unit measured as a maximum platelet aggregation was also converted to percent.
[3]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.
[4]NS = Not significant at $P < 0.05$.

this value. A moderately high positive correlation coefficient of 0.43 was calculated between ADP-induced platelet aggregation and TXB_2 synthesis ($P < 0.05$). There was a negative correlation between ADP-induced platelet aggregation and aortic PGI_2 production. However, the coefficient was statistically insignificant ($r = -0.20$).

Though there were no statistically significant effects, due to large variations among the rats, the highest amount of platelet aggregation was observed in PRP stimulated by collagen (30 ug/ml PRP) from rats fed corn oil without selenium. A moderately high positive correlation coefficient of 0.43 was calculated between collagen-induced platelet aggregation and TXB_2 synthesis and was found to be statistically significant ($P < 0.05$). Though there was a negative correlation between collagen-induced platelet aggregation and PGI_2 production ($r = -0.31$), it was not statistically significant.

Bleeding time. As shown in Table 16, fish oil fed rats (-SeFO and +SeFO) had bleeding times twice as long (19.16 $\pm$ 4.01) as corn oil fed rats (-SeCO and +SeCO) (9.16 $\pm$ 0.86) ($P < 0.001$). There was no statistically significant interaction between the level of dietary selenium and the type of dietary fat. However, bleeding times in rats fed fish oil were 67% longer than that in rats fed corn oil if the intake of selenium was adequate. In contrast, at the level of low dietary selenium, the effect of fish oil feeding on the bleeding time was significant when evaluated

Table 16

Effect of level of dietary selenium and type of dietary fat on bleeding time[1]

Diets[2]	Bleeding time (min)
-SeCO	10.32±1.46
-SeFO	24.99±6.76
+SeCO	7.99±0.80
+SeFO	13.33±3.78
P-values[3]	
Se	NS
Oil	0.02
SexOil	NS

[1]Values are means ± SEM. n = 9 rats per group.
[2]-SeCO = low selenium and corn oil; -SeFO = low selenium and fish oil; +SeCO = high selenium and corn oil; +SeFO = high selenium and fish oil.
[3]NS = Not significant at $P < 0.05$.

by the Student's t-test at $P < 0.05$: bleeding time in rats fed fish oil was 142% longer than that in rats fed corn oil. The fish oil fed rats that were given an inadequate intake of dietary selenium had longer bleeding times than the rats fed fish oil and selenium adequate diets when evaluated by the Student's t-test at $P < 0.05$.

DISCUSSION

The feeding of fish oil increased liver weight and relative liver size in the rats in our experiment, observations which agree with those by other investigators. In a study by Mouri et al. (1984) the level of total lipid and cholesterol in liver was increased by fish oil feeding; in one by Wong et al. (1984) the concentration of hepatic triacylglycerols (TG) was increased by feeding a diet containing 15 wt% fish oil. Both investigators showed a decrease in the concentration of plasma cholesterol and/or serum TG. They suggested that the decreased concentration of circulating TG and/or cholesterol in the fish oil group was associated with the decreased secretion of cholesterol and/or TG by the liver and it was also associated with the decreased synthesis of triacylglycerols and increased oxidation of fatty acids. The increased hepatic weight in the fish oil group in our experiment might be explained partially by the decreased secretion of cholesterol or triacylglycerols. However, the possibility of involvement of fish oil in decreasing the synthesis of and increasing the oxidation of triacylglycerols in the liver was not investigated in this study.

Although there was a profound effect produced on the selenium content of plasma and liver and on the GSHPx activity of plasma, aorta and liver by the level of selenium in the diet, as has been shown repeatedly (Schoene et al.,

1984, 1986; Funk et al., 1987), the feeding of different types of fat produced no significant effects. These observations are similar to those reported elsewhere. Nalbone et al. (1989) fed rats diets that contained either 17 wt% corn oil or 4.5 wt% corn oil and 12.5 wt% fish oil; both diets contained suboptimal levels of selenium (60 ug/kg diet). These diets had a higher fat content and less selenium than those used in our study. Nalbone et al. (1989) showed that hepatic GSHPx activity was the same in both groups of animals.

Although there was no significant effect on the indicators of selenium status (the selenium content of plasma and liver; GSHPx activity in aorta, plasma and liver) when analyzed factorially, all of these variables tended to be lower in the animals fed fish oil than in those fed corn oil. Hepatic GSHPx activity was significantly lower. Taken collectively these data suggest that the feeding of fish oil could lower indicators of selenium status, similar to the lowering of plasma vitamin E that has been shown to occur when fish oils are fed (Nalbone et al., 1988; Mouri et al., 1984). These lower levels did not appear to produce pathophysiologic signs.

The reason that the indicators of selenium status might be lowered by the feeding of fish oils is not known. The selenium in fish is less available than selenium in other foods, even though fish is a good source of this nutrient (Alexander et al., 1983). Dietary fish oil might decrease

the bioavailability of selenium for uptake by tissues and use in the peroxidase.

As n-3 polyunsaturated fatty acids are oxidatively unstable, an increase in membrane unsaturation induced by fish oil intake increases the potential for membrane peroxidation (Labuza, 1971). This increased oxidation is reflected in the MDA content of tissues. The elevation of the level of MDA in the livers from rats fed fish oil supports this concept and agrees with the data of Kobatake et al. (1983) and Mouri et al. (1984). Mouri et al. (1984) noted that as the level of marine oils in the diet increased there was a concomitant increase in thiobarbituric acid reactive substances. However, in our experiment, the increase in the production of MDA that resulted from feeding fish oil was higher in the selenium-deficient animals than in those fed adequate selenium, possibly reflecting an amplification of oxidation by lack of selenium.

The increased level of MDA in the rats fed fish oil could be observed in the aorta too. There was no effect of selenium on the peroxidation in this tissue, however. This may reflect that GSHPx in aorta is less sensitive to selenium deficiency than other tissues (Smith et al., 1973). This conjecture is strengthened by the observation that in our study the activity of GSHPx in the selenium-deficient animals was not depressed in the aorta to the extent that it was in the liver and plasma. It is possible that these elevations could be due to the ingestion of peroxide-rich

oils. Based on the analysis of our diets and those by Kobatake et al.(1983), however, this does not appear to be the case. In our experiment the diets were not higher in peroxides. Although the level of MDA in plasma and serum was increased as a result of fish oil feeding in the studies by Panganamala et al. (1989) and Kobatake et al. (1983), such an increase was not observed in our experiment. The diets in our study contained more than 2 times the amount of vitamin E than was present in the experiment of Kobatake et al. (117 IU vs 50 IU). This higher level of vitamin E in the diet might prevent peroxide levels in plasma from increasing due to fish oil feeding. It does not appear to be suffient, however, to avoid increasing lipid peroxide levels in the aorta and liver.

Unlike earlier work, in our study selenium deficiency did not affect lipid peroxidation to a great extent. Though Masukawa et al. (1983) observed an increase in the level of MDA in aorta from rats fed diets deficient in selenium, in our experiment there was not. The failure to see a significant increase in lipid peroxidation in any tissue except the liver from selenium-deficient animals fed fish oil may be due to the presence of sufficient quantities of dietary vitamin E and other antioxidants such as methionine, vitamin C, copper, and zinc. Hafeman and Hoekstra (1977) also did not see a difference in lipid peroxidation in selenium-deficient rats and suggested that in the presence of a rather high dose of vitamin E (200 IU/ kg), there is

sufficient protection to prevent peroxidation. However, the decreased level of hepatic MDA in selenium supplemented and fish oil fed rats compared to their nonsupplemented counterparts suggests that an adequate supply of selenium is also needed to prevent lipid peroxidation originating from fish oil feeding.

Though the MDA level in the aorta and liver may suggest lipid peroxidation status, it is difficult to relate these MDA values to the lipid peroxide values reported by Moncada et al. (1976b) which influence the conversion of AA to prostacyclin. Maximal stimulation of cyclooxygenase activity will occur at 1 umol/l. A slight increase to only about 1.5 umol/l apparently results in inactivation of prostacyclin synthase and further increase, to 15 umol/l, could result in inactivation of cyclooxygenase as well. Our data for aorta and liver are expressed as nmol MDA/g wet tissue, instead of umol/l. Correlations between these two sets of units and, consequently, physiologically meaningful data are impossible. The level of plasma MDA could, however, be converted to these units and then increased 10-20 fold to account for the poor efficiency of conversion of fatty acid peroxides to MDA. These manipulations indicate that rat plasma contained as much as 20 umol/l, well above the range known to be inhibitory to both cyclooxygenase and prostacyclin synthase. Although these levels are similar to those found in healthy humans, they are high enough to have pathologic consequences. In humans, however, they are not

associated with any known pathology. As discussed by Warso and Lands (1983), the assay loses its credibility when it indicates severe pathology in apparently healthy subjects. They suggest that a specific test for lipid peroxides in biological samples is needed and that this test be applied to the quantification of the peroxide levels in normal and pathological states. Towards this, Lands (1988) has developed an enzymatic assay, using cyclooxygenase, that will measure hydroperoxides in the range of 10 to 200 picomoles. Using this assay he reported that human plasma contained 0.5 uM hydroperoxides, rather than approximately 35 uM that had been measured using the thiobarbituric acid assay. If we had used this assay rather than the MDA test, we might have seen hydroperoxide levels 70 times lower. The approximate 0.3 uM of hydroperoxide would be in the level which could increase the activity of cyclooxygenase but not inhibit the activity of prostacyclin synthase to a great extent.

It is also difficult to relate plasma MDA levels to platelet aggregation. Increased production of MDA has been used as an indicator of prostaglandin production by platelets (Smith et al., 1976). This relationship is sometimes faulty. Panganamala et al. (1989) showed that increased plasma MDA levels were associated with decreased collagen-stimulated platelet aggregation in rats fed fish oil compared to those fed a mixture of corn oil plus lard. Consequently, although MDA can be used as an indication of

peroxidation status, it does not appear to be appropriate to use it as an indicator of prostaglandin synthesis or tendency of platelets to aggregate.

Increased oxidation of fatty acids, as indicated by MDA, can also be reflected by decreases in the concentration of PUFA. Although plasma MDA levels indicated that selenium deficiency caused no differences in lipid peroxidation, its level still affected fatty acid composition. These changes could be seen more easily in plasma than in liver or aorta. The percentage of DHA decreased in plasma and aorta from animals fed the low selenium diet, agreeing with studies by Wittings and Horwitt (1967).

DHA is known to inhibit the synthesis of the 2 series of prostaglandins. Decreases in the content of this fatty acid could possibly lead to increases in the synthesis of TXA_2 and PGI_2. However, since the extent of change in DHA is small, its influence on prostaglandin synthesis is questionable.

In aorta, along with DHA, 22:5 n-3 was decreased in the selenium-deficient groups, especially in those fed fish oil. Though selenium supplementation decreased lipid peroxidation in the liver, its fatty acid composition was not affected. A slight decrease could be observed in AA in plasma. As AA comprised a greater proportion of all the fatty acids than any other n-6 fatty acid, a small change in its level might be important.

The changes in the fatty acid composition of plasma total lipids, aortic, and hepatic phospholipids can be explained in large measure by diet: if the level of a fatty acid increases in a diet, it increases in tissue. Generally the feeding of fish oils led to 1) reductions in the weight percent of members of the n-6 family of fatty acids (18:3, 20:2, 20:4, 22:4, and 22:5) except for 20:3 n-6 and 2) increases in the weight percent of members of the n-3 family of fatty acids (18:3, 20:5, 22:5 and 22:6).

Obviously, however, other effects are occurring because a two fold increase in a dietary component (e.g 18:1 n-9) does not insure similar changes in tissues. The change in weight percent of linoleic acid brought about by the feeding of fish oil was variable and depended on the tissue. The level of LA from the plasma of rats fed fish oil decreased and reflected its level in the diets. In contrast, the level of LA from aorta from rats fed fish oil was higher than that of corn oil fed rats. The difference between the fish oil group and the corn oil group in the level of LA was not observed in the liver. Metabolic modification must also occur. These changes contribute to the modulation in the synthesis of eicosanoids that occurs.

The pattern of change in the levels of the precursors of eicosanoids such as AA and EPA in plasma was similar to those in the phospholipids from aorta and from the liver. For example, an increase in EPA and decrease in AA in the rats fed fish oil occurred in plasma, aorta and liver.

The difference in the ratio of EPA to AA, however, between rats fed fish oil versus those fed corn oil was greater in plasma total lipids, indicating that EPA was more readily incorporated into the plasma lipids than into those of the aorta or liver when fish oil was fed. If the change in the composition of plasma fatty acids represents the change in platelet fatty acid composition as suggested by Croft et al.(1987) and platelets are the site of the production of thromboxane (Herold and Kinsella, 1986), the fact that the composition of fatty acids in the plasma are influenced more by fish oil feeding than other tissues leads to the following interpretation: the reduction in the synthesis of TXA_2 by fish oil feeding will be greater than the change in the synthesis of prostacyclin that occurs in the aorta.

When proportions of EPA and DHA were compared in the three different tissues, EPA was 81%, 69%, and 43% of DHA in plasma, aorta, and liver, respectively. EPA is more slowly incorporated into the liver than into the aorta and plasma. These latter two tissues are the major sites in which the eicosanoids, thromboxane and prostacyclin are synthesized. This suggests that EPA has a greater influence on the synthesis of prostaglandins, as already reported in several studies (Croft et al., 1987). EPA is known to be a poor substrate for cyclooxygenase (Needleman et al., 1979), thus its incorporation into a tissue will lead to a decrease in the synthesis of TXA_2 with a similar changes in the synthesis of PGI_2.

Impaired conversion of 18:2 n-6 to 20:4 n-6 may partly explain the decrease in prostanoid levels observed after fish oil consumption (Kurata and Privett, 1980; Iritani and Narita, 1984 ; Garg et al., 1988b). The last step in the biosynthesis of 20:4 n-6 from 18:2 n-6 in the liver involves the desaturation of 20:3 n-6 by the delta-5 desaturase, an enzyme which is located on the microsomal membrane (Jeffcoat and James, 1984). Garg et al. (1988b) showed that the inclusion of fish oil in the diet at 20 wt% inhibited the conversion of 20:3 n-6 to 20:4 n-6 by the delta 5-desaturase activity in vitro. The increased level of 20:3 n-6 in the plasma, aorta, and liver from the rats fed fish oil in our experiment could be explained by inhibited desaturation of 20:3 n-6 to 20:4 n-6.

The depression in the synthesis of thromboxane with the inclusion of fish oil in the diet correlated well with the decrease in 20:4 n-6 and the increase of 20:5 n-3 in the composition of fatty acids from plasma. In the animals from the fish oil group, due to less available AA, there was decreased synthesis of TXB_2 compared to what was obtained from the corn oil fed group. In addition, there is some possibility that fish oil fed rats produce TXB_3 and PGI_3 by cyclooxygenase and 12-HPEPE by lipoxygenase (Hwang et al., 1988). However, our experiment did not measure the 3 series eicosanoids.

The interaction between selenium and the oils in the synthesis of thromboxane is more difficult to interpret.

At both levels of dietary selenium, fish oil feeding led to decreases in TXB_2 synthesis although the change induced by the selenium-supplemented diet was not as great as that caused by the selenium-deficient ones (58% vs 36%). Why the changes were not similar is not known. The decreased production of TXB_2 synthesis that was present in the fish oil fed animals on the low-selenium diet was accompanied by a similar (59%) decrease in the synthesis of PGI_2. In contrast, while consuming the higher level of dietary selenium, the difference in PGI_2 levels between the fish oil fed group and the corn oil fed group was not significant. Since it is the balance of TXA_2/PGI_2 that is considered when discussing thrombosis and hemostasis, the fact that fish oil feeding did not lower amount of the antiaggregating agent, PGI_2, at the higher level of selenium may become important for maintaining a favorable state. At adequate levels of dietary selenium, the balance may be skewed to favor decreased aggregation; at inadequate levels, this may not be the case.

The increase in the production of PGI_2 in the selenium-deficient animals fed corn oil does not necessarily agree with literature. Toivanen (1987) found that selenium had no effect on the production of PGI_2 by human endothelial cells stimulated with tert-butyl hydroperoxide, a promotor of lipid peroxidation. Other investigators have found that selenium-deficiency inhibits vascular PGI_2 synthesis. The mechanism offered to explain this inhibition is as follows.

In the presence of low GSHPx, 12-HPETE (hydroperoxyeicosatetraenoic acid) produced by lipoxygenase is accummulated and rearranged to THETEs (trihydroxyeicosatetraenoic acids) instead of being reduced to HETE (hydroeicosatetraenoic acid) (Bryant and Bailey, 1980). The increased concentration of THETEs inhibits the synthesis of prostacyclin. Selenium deficiencies in other experiments (Schoene et al., 1986) have been presumed to lead to increased levels of lipid peroxides (up to 1 uM), which would inhibit prostacyclin synthase and activate cyclooxygenase and thromboxane synthase. However, Schoene et al.(1986) did not measure the lipid peroxide level in their experiment to verify the degree of lipid peroxidation and failed to see an increase in THETE in the selenium deficient, corn oil diet. Probably in our experimental system, selenium deficiency did not lead to an excess of lipid peroxidation. If the lipid peroxide level falls below the level necessary to inhibit prostacyclin synthesis, there would be a stimulation of cyclooxygenase, prostacyclin synthase and thromboxane synthase. Due to the greater availability of AA and a slightly increased concentration of peroxides, the rats produced more TXB_2 and PGI_2 in the selenium deficient, corn oil fed group.

Other differences exist between our experiment and those of other investigators, which might explain the contrasting results. They are as follows. First, we used slightly different methods for our assays, especially for

the analysis of 6-keto-PGF_1alpha. Schoene et al. (1986) measured the level of 6-keto-PGF_1alpha in the incubated medium by a bioassay using the degree of inhibition of platelet aggregation. This bioassay is considered to be less specific than the RIA if the sample is not purified (Moore, 1985). In our experiment 6-keto-PGF_1alpha was measured directly by RIA, after dilution with phosphate buffered saline gel and without any extraction. To support our observations Funk et al. (1987) also performed the RIA for PGI_2 and did not observe a decrease in the level of 6-keto-PGF_1alpha when rabbits were fed selenium deficient diets for 7 weeks. Also in the work done by Funk et al. (1987) a 4 week depletion of selenium in the rat showed no difference in 6-keto-PGF_1alpha and it was only after 6 weeks depletion of selenium that there was a decrease in its synthesis. Secondly, the diets contained different types and levels of fat. Thirdly, our diets contained vitamin E at a level more than twice that recommended for rats. This was done because (a) we wanted dietary levels of the vitamin to be the same in all four diets and (b) vitamin E requirements may be higher when diets that contain large amounts of polyunsaturated fatty acids are consumed (Harmon et al., 1966). Often studies that have addressed the role of selenium have failed to indicate or control the level of vitamin E (Masukawa et al., 1983; Schoene et al., 1986).

Once it is established that selenium deficiency promotes the increased production of PGI_2, explanations can

be offered to explain this phenomenon. First, as already mentioned, a slightly increased concentration of peroxides may produce more PGI_2. Second, a greater availability of AA might be caused by selenium. Toivanen (1987) argues that since the production of TXA_2 in a low selenium environment is inhibited less in the presence of exogenous AA, selenium exerts its effect through its action on phospholipase A_2. To further support this contention, one can look to the effect of vitamin E on this enzyme for, in many respects, the behavior of vitamin E and selenium are similar. Panganamala and Cornwell (1982) argue that the activity of phospholipase A_2 is increased in a vitamin E deficient state. In animals fed corn oil and selenium deficient diets, there could be increased phospholipase A_2 activity, providing more substrate for cyclooxygenase and lipoxygenase. Once selenium was added to the corn oil diet the activity of phospholipase A_2 was lessened and with thisdecrease in activity would become lower substrate (AA) concentration. These effects would not be observed in the fish oil diet because they are overpowered by the effect of the n-3 fatty acids. To support this conjecture, a similar trend was seen in the production of TXB_2: it was higher in the selenium-deficient corn oil fed rats than in the selenium-adequate ones. However, this argument is only speculation and requires experimental verification.

In addition to alterations in tissue fatty acids and prostaglandins, dietary fish oils have also been recognized

as affecting aggregability of platelets. Greater availability of AA and the resulting formation of larger amounts of TXB_2 have been implicated in the hypersensitivity of platelets to aggregating agents (ADP and collagen) (Herold and Kinsella, 1986). The fish oil group showed less platelet aggregation induced by ADP than did the corn oil fed group. This observation agreed with the study by Morita et al. (1984). The least aggregation could be observed in the group fed fish oil which had adequate selenium supplementation.

In contrast, collagen-induced aggregation did not show the effect of fish oil feeding in our experiment. Other investigations on fish oil or fish supplements in the diet also indicate no consistent effect on platelet aggregation by collagen (Goodnight et al., 1981; Thorngren et al., 1984; Siess et al., 1981) whereas platelet aggregation by ADP is decreased in human volunteers given salmon oil (Goodnight et al., 1981) or fatty fish such as herring and salmon (Thorngren et al., 1984). Goodnight et al. (1981) saw no response in humans. They reasoned that the high dose of collagen was responsible for the platelets not being responsive to fish oil feeding. The results in the study by Thorngren et al. (1984) were the opposite: a response to collagen was demonstrated. In our experiment, a large variation in collagen-induced platelet aggregation obscured the effectiveness of fish oil or selenium supplements in modifying this variable. The tendency, however, was towards

having the greatest amount of platelet aggregation in the group fed corn oil, without selenium supplementation.

Though the effect of selenium on platelet aggregation was not significant in our experiment, the observation that the least ADP-induced aggregation was seen in the selenium sufficient and fish oil fed group and the most collagen-induced aggregation was seen in the selenium deficient and corn oil fed group supported the theory that adequate selenium in diet might help decrease platelet aggregation. Masukawa et al. (1983) and Schoene et al. (1986) showed a significant decrease in platelet aggregation when diets were supplemented with selenium. Wang and Kiem (1988), however, saw no significant relationship between selenium and platelet aggregation.

Fish oil feeding prolonged tail bleeding time, confirming observations in the study by Hornstra et al. (1981). However, German et al. (1985) failed to see the lengthening effect of fish oil on bleeding time even at a high percentage of fish oil in the diet. This could be due to their short feeding period (3 weeks compared to our 8 weeks) and large individual variation in the measurement of this variable.

Schiavon et al.(1984) observed increased bleeding time when selenium supplements were given to healthy human subjects who had a normal selenium status. Unexpectedly, in our experiment the selenium-supplemented rats showed shorter bleeding times than the selenium-depleted rats. Extremely

prolonged bleeding times were observed in several rats on the low selenium diets. Bleeding times are regulated by mechanisms in addition to the TXB_2 and PGI_2 balance. The animals had a pronounced nutritional deficit and all physiologic manifestations of this are not known.

Overall, fish oil feeding appeared to lower indicators of selenium status. Selenium-deficiency did not affect lipid peroxidation caused by fish oil feeding to a great extent although selenium supplementation may have slightly improved lipid peroxidation in the liver. Though selenium supplementation increased the level of n-3 fatty acid (such as 22:6 n-3) in plasma and the aorta, its overall effect on the precursors of eicosanoids was smaller than the effect of fish oil feeding. Fish oil feeding decreased the level of 20:4 n-6 and increased the level of 20:5 n-3 in plasma, aorta, and liver to a great extent. It also led to a reduction in the synthesis of TXB_2. Though the effect of selenium supplementation on fatty acid composition and TXB_2 synthesis was weaker than the effect of fish oil on these two variables, the effect of selenium supplementation on synthesis of PGI_2 was strong enough to keep its level similar in the selenium-supplemented rats regardless of the type of fat. The ADP-induced platelet aggregation was least in the selenium adequate and fish oil group. The bleeding time was significantly decreased by the fish oil feeding. Selenium-deficiency increased bleeding time unexpectedly. Overall, the beneficial effects of selenium supplementation

on the synthesis of prostaglandins and hemostatic functions were weaker than the effects of fish oil. Thus, the suggestion by Thorngren et al. (1987) that the selenium content of fish, instead of EPA, is the source of its therapeutic value has not been substantiated by this study. Selenium might contribute but the magnitude of its significance is questioned.

SUMMARY AND CONCLUSION

The purpose of this study was to investigate whether the beneficial effects which the consumption of fish oil imparts on hemostatic function can be modified by the level of dietary selenium. Male Sprague Dawley rats were fed for 8 weeks semipurified diets containing 7 wt% corn oil or 5.5 wt% "MaxEPA" fish oil plus 1.5 wt% corn oil with or without selenium supplementation (0.5 mg/kg of diet). Effects of the diets on selenium status (i.e., selenium content of tissues and selenium-dependent GSHPx), malondialdehyde (MDA) levels and fatty acid composition of plasma, aorta, and liver were examined. Thromboxane B_2 synthesis during platelet aggregation induced by collagen and 6-keto-PGF_1alpha synthesis by incubated aorta were measured. To evaluate hemostatic function, ADP- and collagen-induced platelet aggregation and tail bleeding time were measured.

The major conclusions of this study are as follows.

1) The feeding of fish oil lowered indicators of selenium status as indicated by hepatic GSHPx activity.

2) The feeding of fish oil increased the level of MDA in the liver and aorta. Supplementation of the diets with selenium decreased MDA levels only in the liver of those animals fed fish oil.

3) In the animals that were selenium deficient, 22:6 n-3 levels in aorta and plasma were decreased, especially in the fish oil group.

4) The feeding of fish oil decreased 20:4 n-6 and increased 20:5 n-3 in plasma total lipids, aortic and hepatic phospholipids and increased the level of 20:3 n-6 in all tissues. This increase in the content of 20:3 n-6 may indicate that delta 5-desaturase activity was decreased by fish oil feeding.

5) The depression in the level of TXB_2 with the inclusion of fish oil in the diet correlated well with the decrease in 20:4 n-6 and the increase of 20:5 n-3 in the composition of fatty acids from plasma.

6) Selenium supplementation tended to decrease TXB_2 level in the corn oil group. Selenium deficiency led to an increase in the synthesis of 6-keto-PGF_1alpha in the rats fed low selenium and corn oil. Selenium supplementation eliminated the difference in the level of 6-keto-PGF_1alpha between fish and corn oil fed groups.

7) Fish oil decreased ADP-induced platelet aggregation significantly; selenium supplementation tended to decrease it. Neither treatment changed collagen-induced platelet aggregation although it tended to be highest in the corn oil-fed rats without added selenium.

8) Although the feeding of fish oil significantly increased bleeding time, selenium supplementation did not.

These conclusions indicate that selenium supplementation may decrease fish oil-induced lipid peroxidation in liver and may prevent oxidation of 22:6 n-3 fatty acid of tissue. As suggested by Warso and Land (1983)

a more specific test for lipid peroxides in biological samples and its application to the quantitation of the peroxide levels in normal and pathophysiological states should be developed. Selenium supplementation may decrease TXB_2 synthesis and ADP-induced platelet aggregation but not bleeding time. The beneficial effects of selenium supplementation on hemostatic function are very small compared to the effect of fish oil feeding.

Though phospholipase is suggested to be affected by dietary selenium levels, it must be measured directly instead of deducing its activity by increased PGI_2 and TXB_2 synthesis in animals fed the low selenium with corn oil diet.

Components other than selenium and EPA may play a role in the therapeutic value of fish because, as Kromhout et al. (1985) suggested, the amount of EPA in fish is not enough to explain the beneficial effect on CVD and selenium has less of a beneficial effect than EPA in our experiment.

BIBLIOGRAPHY

Aharony, D., Smith, J. B. & Silver, M. J. (1982) Regulation of arachidonate induced platelet aggregation by the lipoxygenase product, 12-hydroperoxyeicosatetraenoic acid. Biochem. Biophys. Acta. 718: 193-200.

Alexander, A. R., Whanger, P. D. & Miller, L. T. (1983) Bioavailability to rats of selenium in various tuna and wheat products. J. Nutr. 113: 196-204.

American Institute of Nutrition (1977) Report of the American Institute of Nutrition Ad Hoc Committee on standards for nutritional studies. J.Nutr. 107: 1340-1348.

Bang, H. O., Dyerberg, J. & Hjorne, N. (1976) The composition of food consumed by Greenland Eskimos. Acta. Med. Scand. 200: 69-73.

Bang, H.O. & Dyerberg, J. (1980) Lipid metabolism and ischemic heart disease in Greenland Eskimos. Adv. Nutr. Res. 3: 1-22.

Beilstein, M. A. & Whanger, P. D. (1986) Deposition of dietary organic and inorganic selenium in rat erythrocyte proteins. J. Nutr. 116: 1701-1710.

Bell, R. L., Kennerly, D. A., Stannford, N. & Majerus, P. W. (1979) Diglyceride lipase; a pathway for arachidonate release from human platelets. Proc. Natl. Acad. Sci. USA 76: 3238-3241.

Bieri, J. G. & Andrew, E. L. (1963) Metabolic effects of selenium as related to vitamin E. J. A. O. C. S. 40: 365-368.

Bieri, J. G. & Andrew, E. L. (1964) Fatty acids in rat testes as affected by vitamin E. Biochim. Biophys. Res. Commun. 17: 115-119.

Billah, M. M., Lapetina, E. G. & Cuatrecasa, P. (1979) Phosphatidylinositol-specific phospholipase-C of platelets; association with 1,2-diacylglycerol-kinase and inhibition by cyclic-AMP. Biochem. Biophys. Res. Commun. 90: 92-98.

Bills, T. K., Smith, J. B. & Silver, M.J. (1976) Metabolism of [^{14}C] arachidonic acid by human platelets. Biochem. Biophys. Acta. 424: 303-314.

Bligh, E. G. & Dyer, W. J. (1959) A rapid method of total lipid extraction and purification. Canadian J. Biochem. and Physio. 37: 911-917.

Born, G. V. R. & Cross, M. J. (1963) Effect of adenosine diphosphate on concentration of platelets in circulating blood. Nature 197: 974-976.

Boukhchache, D. & Lagarde, M. (1982) Interactions between prostaglandin precursors during their oxygenation by human platelets. Biochem. Biophys. Acta 713: 386-392.

Brown, M. W. & Watkinson, J. H. (1977) An automated fluorimetric method for the determination of nanogram quantities of selenium. Analytica Chimica Acta 89: 29-35.

Brox, J. H., Killie, J. E., Gunnes, S., and Nordoy, A. (1981) The effect of cod liver oil and corn oil on platelets and vessel wall in man. Thromb. Haemost. 46: 601-611.

Bruckner, G. G., Lokesh, B., German, J. B. & Kinsella, J. E. (1984) Biosynthesis of prostanoids, tissue fatty acid composition and thrombotic parameters in rats fed diets enriched with docosahexaenoic (22:6 n3) or eicosapentaenoic (20:5 n3) acids. Thrombosis Res. 34: 479-497.

Bryant, R. W. & Bailey, M. (1980) Altered lipoxygenase metabolism and decreased glutathione peroxidase activity in platelets from selenium deficient rats. Biochem. Biophys. Res. Comm. 92: 268-276.

Bunting, J., Gryglewski, R., Moncada, S., & Vane, J.R. (1976) Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. Prostaglandins 12: 897-913.

Burk, R. F. (1976) Selenium in man. In: Trace elements in human health and disease. Vol.II. Essential and toxic elements. (Prasad, A.S.& Oberleas, D.eds.), pp.105-133 Academic Press NY.

Burk, R. F., Lawrence, R. A. & Correia, M. A. (1980) Sex differences in biochemical manifestations of selenium deficiency in rat liver with special reference to heme metabolism. Biochem. Pharmacol. 29: 39-42.

Carmagnol, R., Sinet, P.M. & Jerome, H. (1983) Selenium-dependent and non-selenium-dependent glutathione

peroxidases in human tissue extracts. Biochim. Biophys. Acta. 759: 49-57.

Chan, A. C. (1987) Influence of dietary vitamin E on eicosanoid synthesis. In: Clinical and nutritional aspects of vitamin E. (Hayaishi, O. & Mino, M. eds.), pp.. 309-311, Elsevier Science Publishers B.V., Amsterdam, Netherland

Cook, H. W. & Lands, W. E. M. (1976) Mechanism for suppression of cellular biosynthesis of prostaglandins. Nature 260: 630-632.

Croft, K. D., Beilin, L. J., Vandongen, R. & Mathews, E. (1984) Dietary modification of fatty acid and prostaglandin synthesis in the rat. Biochim. Biophys. Acta 795: 196-207.

Croft, K. D., Codde, J. P., Barden, A., Vandongen, R. & Beilin, L. J. (1985) Onset of changes in phospholipid fatty acid composition and prostaglandin synthesis following dietary manipulation with n-6 and n-3 fatty acids in the rat. Biochim. Biophy. Acta 834: 316-323.

Croft, K. D., Beilin, L. J., Legge, F. M. & Vandongen, R. (1987) Effects of diets enriched in eicosapentaenoic or docosahexaenoic acids on prostanoid metabolism in the rat. Lipids 22: 647-650.

Croset, M. & Lagarde, M. (1983) Stereospecific inhibition of PGH_2-induced platelet aggregation by lipoxygenase products of icosanoic acids. Biochem. Biophys. Res. Commun. 112: 878-883.

Croset, M. & Lagarde, M. (1985) Enhancement of eicosanoic acid lipoxygenation in human platelets by 12-hydroperoxy derivative of arachidonic acid. Lipids 20: 743-750.

Culp, B. R., Titus, B.G. & Lands, W. E. M. (1979) Inhibition of prostaglandin biosynthesis by eicosapentaenoic acid. Prostaglandin Med. 3: 269-278.

Deagen, J. T., Butler, J. A., Beilstein, M. A. & Whanger, P. D. (1987) Effects of dietary selenite, selenocysteine and selenomethionine on selenocysteine lyase and glutathione peroxidase activities and on selenium levels in rat tissues. J. Nutr. 117: 91-98.

DeSchrijver, R. & Privett, O. (1982) Effects of dietary long-chain fatty acids on the biosynthesis of unsaturated fatty acids in the rat. J.Nutr. 112: 619-626.

Diczfalusy, U., Falardeau, P. & Hammarstrom, S. (1977) Conversion of prostaglandin endoperoxides to C^{17}-hydroxy acids catalyzed by human platelet thromboxane synthase. FEBS letter 84: 271-274.

Doni, M. G., Bonaccorso, G. & Piva, E. (1983) High glutathione peroxidasse and prostacyclin-like activity generation in rat aorta. Hemostasis 13: 248-253.

Dyerberg, J., Jorgensen, K. A. & Arnfred, T. (1981) Human umblical blood vessel converts all cis-5,8,11,14,17 eicosapentaenoic acid to prostaglandin I_3. Prostaglandins 22: 857-862.

Egan, R. W., Tischler, A. N., Baptista, E. M., Ham, E. A., Soderman, D. D. & Gale, P. H. (1983) Specific inhibition and oxidative regulation of 5-lipoxygenase. In: Advances in prostaglandin, thromboxane, and leukotriene research. Vol 11 (Samuelsson, B., Paoletti, R., Ramwell, P. eds), pp. 151-157, Raven Press, New York

Farrell, P. M. (1988) Vitamin E. In: Modern nutrition in health and disease. (Shils, M. E. & Young, V.R., eds.), pp.340-354, 7th ed., Lea & Febiger, Philadelphia

Ferreti, A., Judd, J., Marshall, M., Flanagan, V.& Matusik, E. (1985) Moderate changes in linoleate intake do not influence the systemic production of E prostaglandins. Lipids 20: 268-272.

Fischer, W.C. & Whanger, P. D. (1977) Fatty acid and glucose metabolism in selenium deficient rats and lambs. J. Nutr. 107: 1493-1501.

Fischer, S. & Weber, P. (1983) Thromboxane A_3 (TXA_3) is formed in human platelets after dietary eicosapentaenoic acid (C20:5w-3). Biochem. Biophys. Res.Comm. 116: 1091-1099.

Fischer, S. & Weber, P. (1984) Prostaglandin I_3 is formed in vivo in man after dietary eicosapentaenoic acid. Nature 307: 165-168.

Food and Nutrition Board, National Research Council (1980) Recommended Dietary Allowances. 9th ed., pp.162-163, National Academy of Sciences, Washington, DC.

Fritsche, K. L. & Johnston, P. V. (1988) Rapid autoxidation of fish oil in diets without added antioxidants. J. Nutr. 118: 425-426.

Funk, C. D. & Powell, W. S. (1985) Release of prostaglandins and monohydroxy and trihydroxy

metabolites of linoleic and arachidonic acids by adult and fetal aortae and ductus arteriosus. J. Biol. Chem. 260: 7481-7488.

Funk, C. D., Boubez, W. & Powell, W. S. (1987) Effects of selenium-deficient diets on the production of prostaglandins and other oxygenated metabolites of arachidonic acid and linoleic acid by rat and rabbit aortae. Biochim. Biophys. Acta 921: 213-220.

Ganther, H.E., Hafeman, D. G., Lawrence, R. W. Serfass, R. E., & Hockstra, W. G. (1976) Selenium and GSHPx in health and disease. In: Trace elements in human health and disease (Prasad, A. S., Oberleas, D. eds.) Vol. 2., pp.165-234, Nutrition Foundation Academic Press, NY

Garg, M. L., Sebokova, E., Thomson, B. R.& Clandinin, M. T. (1988a) 6-desaturase activity in microsomes of rats fed diets enriched with cholesterol and/or w-3 fatty acids. Biochem. J. 249: 351-356.

Garg, M., Thomson, B. R. & Clandinin, M. T. (1988b) Effect of dietary cholesterol and/or w-3 fatty acids on lipid composition and delta 5-desaturase activity of rat liver microsomes. J. Nutr. 118: 661-668.

Garg, M. L., Wierzbicki, A., Keelan, M., Thomson, A. B. R. & Clandinin, M. T. (1989) Fish oil prevents changes in arachidonic acid and cholesterol content in rat caused by dietary cholesterol. Lipids 24: 266-270.

German, J. B., Lokesh, B., Bruckner, G. G. & Kinsella, J. E. (1985) Effect of increasing levels of dietary fish oil on tissue lipids and prostaglandin synthesis in the rat. Nutr. Res. 5: 1393-1407.

Gerrard, J. M., Graff, G., Dedon, P. C., Kindom, S. E. & White, J. G. (1982) 1-Arachidonyl-monoglyceride causes platelet aggregation-implications for an acylglycerol acylhydrolase involvement in control of prostaglandin synthesis. Prog. Lipid Res. 20: 575-578.

Gerrard, J. M. (1985) Hemostasis, thrombosis, and atherosclerosis; The response of blood cells to vessel wall injury. In: Prostaglandins and leukotrienes; Blood and vascular cell function., pp.217-238, Marcel Dekker Inc. N.Y.

Goodnight, S., Harris, W. & Connor, W. (1981) The effects of dietary w3 fatty acids on platelet composition and function in man: A prospective, controlled study. Blood 58: 880-885.

Gordon, P. R. & O'Dell, B. L. (1980) Short-term zinc deficiency and homeostasis in the rat. Proc. Soc. Exp. Biol. Med. 163: 240-244.

Granstrom, E. (1986) Methodology in prostaglandin and thromboxane assay. Pro. Lipid. Res. 25: 119-127.

Granstrom, E. (1987) Biochemistry of the eicosanoids: cyclooxygenase and lipoxygenase products of polyunsaturated fatty acids. In: Lipids in modern nutrition. (Horisberger, M. & Bracco, U. eds.), pp. 59-66, Nestle Nutrition, Velvey/Raven Press, NY

Granstrom, E. & Kumlin, M. (1987) Metabolism of prostaglandins and lipoxygenase products; relevance for eicosanoid assay. In: Prostaglandins and related substances: a practical approach. (Benedetto, C., McDonald-Gibson, R. G., Nigam, S. & Slater, T. f. eds.), pp.5-27, IRL Press, Ltd., Oxford, England

Greenwald, J. E., Bianchine, J. R. & Wong, L. K. (1979) The production of the arachidonate metabolite HETE in vascular tissue. Nature 281: 588-589.

Gryglewski, R., Bunting, S., Moncada, S., Flower, R. J. & Vane, J. R. (1976) Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. Prostaglandins 12: 685-713.

Guidi, G., Schiavon, R., Sheiban, I. & Perona, G. (1986) Platelet glutathione peroxidase activity is impaired in patients with coronary heart disease. Scand J. Clin. Lab. Invest. 46: 549-551.

Hafeman, D. G. & Hoekstra, W. G. (1977) Lipid peroxidation in vivo during vitamin E and selenium deficiency in the rat as monitored by ethane evolution. J.Nutr. 107: 666-672.

Hamazaki, T., Hirai, A., Terano, T., Sajiki, J., Kondo, S., Fugita, T., Tamura, Y. & Kumagai, A. (1982) Effects of orally administated ethylester of eicosapentaenoic acid on PGI2-like substance production by rat aorta. Prostaglandins 23: 557-567.

Hamberg, M. & Samuelsson, B. (1967) The specificity of the oxygenation of unsaturated fatty acid. J. Biol. Chem. 242: 5329-5335.

Hamberg, M. J., Svensson, J., Wakabayashi, T. & Samuelsson, B. (1974) Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. Proc. Natl. Acad. Sci. USA 71: 345-349.

Hamberg, M. J., Svensson, J. & Samuelsson, B. (1975) Thromboxanes; A new group of biologically active compounds derived from prostaglandin endoperoxides. Proc. Natl. Acad. Sci. USA 72: 2994-2998.

Harmon, E. M., Witting, L. A. & Horwitt, M. K. (1966) Relative rates of depletion of alpha-tocopherol and linoleic acid after feeding polyunsaturated fats. Am. J. Clin. Nutr. 18: 243-248.

Harris, W. S. (1989) Fish oil and plasma lipid and lipoprotein metabolism in humans; a critical review. J.Lipid Res. 30:785-807.

Herman, A. G., Claeys, M., Moncada, S. & Vane, J. R. (1979) The production of prostacyclin (PGI_2) and 12-hydroxy-5,18,10,14-eicosatetraenoic acid (HETE) by pericardium, pleura, peritoneum and aorta of the rabbit. Prostaglandins 18:439-452

Herold, P. M., & Kinsella, J. E. (1986) Fish oil consumption and decreased risk of cardiovascular disease; a comparison of findings from animal and human feeding trials. Am. J. Clin. Nutr. 43: 566-578.

Hornstra, G. (1982) "Dietary Fats, Prostanoids and Arterial Thrombosis" Martinus Nijhoff Publishers, Boston

Hornstra, G., Christ-Hazelhof, E., Haddeman, E., Ten Hoor, F. & Nuggtern, D. H. (1981) Fish oil feeding lowers thromboxane and prostacyclin production by rat platelets and aorta and does not result in the formation of prostaglandin I_3. Prostaglandins 21: 727-738.

Horwitt, M. K. (1965) Role of vitamin E, selenium, and polyunsaturated fatty acids in clinical and experimental muscle disease. Fed. Proc. 24: 68-92.

Hoskin, R. L. & Putnam, J. J. (1987) Oil crops: situation and outlook report. Oct 15,1987

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: 590-597.

Hwang, D. H. (1982) Characteristics of the formation of the platelet lipoxygenase product from endogenous arachidonic acid. Lipis 17: 845-847.

Hwang, D.H., Chanmugam, P. & Wheeler, C. (1984) Zinc deficiency affects neither platelet arachidonic acid

metabolism nor platelet aggregation in rats. J. Nutr. 114: 398-403.

Hwang, D. H., Boudreau, M. & Chanmugam, P. (1988) Dietary linoleic acid and longer-chain n-3 fatty acids; comparison of effects on arachidonic acid metabolism in rats. J. Nutr. 118: 427-437.

Hwang, D. H. (1989) Essential fatty acids and immune response. FASEB J. 3: 2052-2061.

Iritani, N. & Fujikawa, S. (1982) Competitive incorporation of dietary w-3 and w-6 polyunsaturated fatty acids into the tissue phospholipids in rats. J. Nutr. Sci. Vitaminol. 28: 621-629.

Iritani, N. & Narita, R. (1984) Changes of arachidonic acid and n-3 polyunsaturated fatty acids of phospholipid classes in liver, plasma, and platelets during dietary fat manipulation. Biochim. Biophy. Acta 793: 441-447.

IUPAC-IUB (1977) The nomenclature of lipids. Eur. J. Biochem. 79: 11-21.

Jeffcoat, R. & James, A. T (1984) The regulation of desaturation and elongation of fatty acids in mammals. In: Fatty acid metabolism and its regulation. (Numa S. ed.), pp.85-112, Elsevier Science Publishers B.V., Amsterdam, Netherlands

Jensen, G. E. & Clausen, J. (1981) Glutathione peroxidase activity in vitamin E and essential fatty acid-deficient rats. Ann. Nutr. Metab. 25: 27-37.

Kawaguchi, H., Ishibashi, T. & Imai, Y. (1982) Increased thromboxane B2 biosynthesis in platelets. Lipids 17: 577-584.

Ke, P. J., Cervantes, E. & Robles-Martinez, C. (1984) Determination of thiobarbituric acid reactive substances in fish tissue by an improved distillation spectrophotometric method. J. Sci. Food Agric. 35: 1248-1254.

Kent, R. S., Dietrich, S. L. & Whorton, A. R. (1983) Regulation of vascular prostaglandin synthesis by metabolites of arachidonic acid in perfused rabbit aorta. J. Clin. Invest. 72: 455-465.

Kinsella, J. E. (1986a) Food components with potential therapeutic benefits; the n-3 polyunsaturated fatty acids of fish oils. Food Tech. 40(2): 89-97, 146.

Kinsella, J. E. (1986b) Dietary fish oils: Possible effects of n-3 polyunsaturated fatty acids in reduction of thrombosis and heart disease. Nutr.Today 21 Nov./Dec. : 7-14.

Kobatake, Y., Hirahara, F., Innami, S. & Nishide, E. (1983) Dietary effect of w3 type polyunsaturated fatty acids on serum and liver lipid levels in rats. J. Nutr. Sci. Vitaminol. 29: 11-27.

Kromhout, D., Bosschieter, E.B., DeLezenne-Coulander, C. (1985) The inverse relationship between fish consumption and 20 years mortality from coronary heart disease. N. Eng. J. Med. 312: 1205-1209.

Kurata, N. & Privett, O. (1980) Effect of dietary fatty acid composition on the biosynthesis of unsaturated fatty acids in the rat liver microsome. Lipids 15: 512-518.

Labuza, T. P. (1971) Kinetics of lipid oxidation in foods. CRC Critical Reviews in Food Tech. 2: 355-405.

Lagarde, M. (1988) Metabolism of fatty acids by platelets and the functions of various metabolites in mediating platelet function. Prog. Lipid Res. 27: 135-152.

Lands,W. E. M. (1979) The biosynthesis and the metabolism of prostaglandins. Ann. Rev. Physiol. 41: 633-652.

Lands, W. E. M. (1986) "Fish and Human Health". Academic Press, New York

Lands, W. E. M. (1988) Peroxide tone in regulating eicosanoid formation. In: Lipid peroxidation in biological systems. (Sevanian, A. ed.), pp.155-162, American Oil Chemists' Society, Champaign, Ill

Lawrence, R. A., Parkhill, L. K. & Burk, R. F. (1978) Hepatic cytosolic non-selenium-dependent glutathione peroxidase activity: Its nature and effect of selenium deficiency. J. Nutr. 108: 981-987.

Levander, O. A., Surtherland, B., Morris, V. C. & King, J. C. (1981) Selenium metabolism in human nutrition. In: Selenium in biology and medicine. (Spallholz, J. E. Martin, J. L. & Ganther, H. E. eds.) AVI Publ. Co., Westport, CONN

Lockette, W. E., Webb, R. C., Culp, B. R. & Pitt, B. (1982) Vascular reactivity and high dietary eicosapentaenoic acids. Prostaglandins 24: 631-639.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Machlin, L. A. & Bendich, A. (1987) Free radical tissue damage: protective role of antioxidant nutrients. FASEB J. 1: 441-445.

Marcus, A. J. (1984) The eicosanoids in biology and medicine. J. Lipid Res. 25: 1511-1516.

Marshall, P. J., Kulmacz, R. J., & Lands, W. E. M. (1987) Constraints on prostaglandin biosynthesis in tissues. J. Biol. Chem. 262: 3510-3517.

Marx, J. L. (1982) The leukotrienes in allergy and inflammation. Science 215: 1380-1383.

Masukawa, T., Goto, J. & Iwata, H. (1983) Impaired metabolism of arachidonate in selenium deficient animals. Experientia 39: 405-406.

Mertz, W. (1981) The essential trace elements. Sci. 213: 1332-1338.

Mitchell, L. L., Allen, K. G. D. & Mathias, M. M. (1988) Copper deficiency depresses rat aortae superoxide dismutase activity and prostacyclin synthesis. Prostaglandins 35: 977-986.

Moncada, S. & Vane, J. R. (1978) Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2, and prostacyclin. Pharmacol. Rev. 30: 293-331.

Moncada, S. & Vane , J. R. (1979) Arachidonic acid metabolites and the interactions between platelets and blood vessel walls. N. Eng. J. Med. 300: 1142-1147.

Moncada, S., Needleman, P., Bunting, S. & Vane, J. R. (1976a) Prostaglandin endoperoxides and thromboxane generating systems and their selective inhibition. Prostaglandins 12: 323-336.

Moncada, S., Gryglewski, R. J., Bunting, S. & Vane, J. R. (1976b) A lipid peroxide inhibits the enzyme in blood vessel microsomes that generates from prostaglandin endoperoxides the substance (prostaglandin X) which prevents platelet aggregation. Prostaglandins 12: 715-737.

Moore, P. K. (1985) Biosynthesis and catabolism of prostaglandins, thromboxanes and leukotrienes. In: Prostanoids: pharmacological, physiological and

clinical relevance., pp. 1-40, Cambridge University Press, Cambridge, London

Morita, I., Komeshima, N., Takahashi, R., Orimo, H. & Murota, S. (1984) A proposed method for exploring antiaggregatory effects of eicosapentaenoic acid in the rat. Prostaglandins Leukotriens Med. 14: 123-129.

Morris, V. C. & Levander, O. A. (1970) Selenium content of foods. J. Nutr. 100: 1383-1388.

Morrison, W. R. & Smith, L. M. (1964) Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5: 605-608.

Mouri, K., Ikesu, H., Esaka, T. & Igarashi, O. (1984) The influence of marine oil intake upon levels of lipids, alpha-tocopherol and lipid peroxidation in serum and liver of rats. J. Nutr. Sci. Vitaminol. 30: 307-318.

Mutanen, M. L. & Mykkanen, H. M. (1984) Effect of dietary fat on plasma glutathione peroxidase levels and intestinal absorption of ^{75}Se-labeled sodium selenite in chicks. J. Nutr. 114: 829-834.

Nalbone, G., Leonardi, J., Termine, E., Portugal, H., Lechene, P., Pauli, A-M., and Lafont, H. (1989) Efects of fish oil, corn oil and lard diets on lipid peroxidation status and glutathione peroxidase activities in rat heart. Lipids 24: 179-186.

Nalbone, G., Termine, E., Leonardi, J., Portugal, H., Lechene, P., Calaf, R., Lafont, R. & Lafont, H. (1988) Effect of dietary salmon oil feeding on rat heart lipid status. J. Nutr.118: 809-817.

Nassar, B. A., Huang, Y. S. & Horrobin, D. F. (1986) Response of tissue phospholipid fatty acid composition to dietary n-6 and replacement with marine n-3 and saturated fatty acids in the rat. Nutr. Res. 6: 1397-1409.

Needleman, P., Moncada, S., Bunting, S., Vane , J. R., Hamberg, M. & Samuelsson, B. (1976) Identification of an enzyme in platelet microsomes which generates thromboxane A_2 from prostaglandin endoperoxides. Nature 261: 558-560.

Needleman, P., Raz, A., Minkes, M. S., Ferrendelli, J. A. & Sprecher, H. (1979) Triene prostaglandins: Prostacyclin and thromboxane biosynthesis and unique biological properties. Proc. Natl. Acad. Sci. USA 76:944-948.

Nelson, G. J. & Ackman, R. G. (1988) Absorption and transport of fat in mammals with emphasis on n-3 polyunsaturated fatty acids. Lipids 23: 1005-1014.

Nicosia, S. & Patron, C.(1989) Eicosanoid biosynthesis and action; novel opportunities for pharmacological intervention. FASEB J. 3: 1941-1948.

Ohkawa, H., Ohishi, N. & Yagi, K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95: 3351-358.

Paglia, D. E. & Valentine, W. N. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70: 158-169.

Panganamala, R. V. & Cornwell, D. G. (1982) The effects of vitamin E on arachidonic acid metabolism. Ann. New York. Acad. Sci. 393: 376-391.

Panganamala, R. V., Cornwell, D. G., Davis, W. B., Stone, D. W. & Feng, Y. M. (1989) The effect of menhaden oil (MO) on platelet aggregation (PA), plasma malonadehyde (MDA) and plasma vit E (VE) in the rabbit. FASEB J. 3: A950, ABS # 4208

Pendell, H. W., Muth, O. H., Oldfield, J. E. & Weswig, P. H. (1969) Tissue fatty acids in normal and myopathic lambs. J. Animal Sci. 29: 94-98.

Peterson, D.A., Gerrard, J.M., Peller, J., Rao, G.H.R., and White, J.G. (1981) Interaction of zinc and arachidonic acid. Prostaglandins and Med. 6: 91-99.

Powell, W. S. and Funk, C.D. (1987) Metabolism of arachidonic acid and other polyunsaturated fatty acids by blood vessels. Prog. Lipid Res. 26: 183-210.

Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G. & Hoekstra, W. G. (1973) Selenium: Biochemical role as a component of glutathione peroxidase. Sci. 179: 588-590.

Salmon, J. A., Smith, D. R., Flower, R. J., Moncada, S. & Vane, J. R. (1978) Further studies on enzymatic conversion of prostaglandin endoperoxide into prostacyclin by porcine aorta microsomes. Biochim. Biophys. Acta 523: 250-262.

Salonen, J. T., Alfthan, G., Huttunen, J. K., Pikkarainen, J. & Puska, P. (1982) Association between cardiovascular death and myocardial infarction in a matched pair longitudinal study. Lancet ii: 175-179.

Salonen, J. T. (1989) Antioxidants and platelets. Annals of Medicine 21: 59-62.

Sanders, T. A. B. & Roshanai, F. (1983) The influence of different types of w3 polyunsaturated fatty acids on blood lipids and platelet function in healthy volunteers. Clin. Sci. 64: 91-99.

Satoh, K. (1978) Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chemica Acta 90: 37-43.

Schiavon, R., Freeman, G.E., Guidi, G.C., Perona, G., Zatti, M., and Kakkar, V.V. (1984) Selenium enhances prostacyclin production by cultured endothelial cells: possible explanation for increased bleeding times in volunteers taking selenium as a dietary supplement. Thrombosis Res. 34: 389-396.

Schoene, N. W., Morris, V. C. & Levander, O. A. (1984) Effects of selenium deficiency on aggregation and thromboxane formation in rat platelets. Fed. Proc. 43: 477 (Abs. No. 1125)

Schoene, N. W., Morris, V. C. & Levander, O. A. (1986) Altered arachidonic acid metabolism in platelets and aortas from selenium-deficient rats. Nutr. Res. 6: 75-83.

Schwarz,K. (1961) Development and status of experimental work on factor 3-selenium. Fed. Proc. 20 : 666-673.

Serhan, C. N., Hamberg, M. & Samuelsson, B. (1984) Lipoxins: Novel series of biologically active compounds formed from arachidonic acid in human leukocytes. Proc. Natl. Acad. Sci. 81: 5335-5339.

Seyberth, H., Oelz, O., Kennedy, T., Sweetman, B., Danon, A., Frolich, J. C., Heimberg, M. & Oates, J. A. (1975) Increased arachidonate in lipids after administration to man: Effects on prostaglandin biosynthesis. Clinical Pharmacology and Therapeutics 18: 521-529.

Siess, W., Roth, P. & Weber, P. C. (1981) Stimulated platelet aggregation, thromboxane B_2 formation and platelet sensitivity to prostacyclin - A critical evaluation. Thrombosis and Haemostasis 45: 204-207.

Siess, W., Roth, P., Scherer, B., Kurzman, I., Bolig, B. & Weber, P.C. (1980) Platelet-membrane fatty acids, platelet aggregation, and thromboxane formation during mackerel diet. Lancet i : 441-444.

Silver, M. J., Hoch, W., Kocsis, J. J., Ingerman, C. M. & Smith, J. B. (1974) Arachidonic acid causes sudden death in rabbits. Science 183: 1085-1087.

Smith, A. G., Harland, W. A.& Brooks, C. J. W. (1973) Glutathione peroxidase in human and animal aortas. Steroids Lipid Res. 4: 122-128.

Smith, J. B., Ingerman, C. M. & Silver, M. J. (1976) Malondialdehyde formation as an indicator of prostaglandin production by human platelets. J. Lab. Clin. Med. 88: 167-172

Snedecor,G. W. & Cochran,W. G. (1980) Statistical Methods. 7th ed. Iowa State Press Iowa.

Socini, A., Galli, C., Colombo, C.& Tremoli, E. (1983) Fish oil administration as a supplement to a corn oil containing diet affects arterial prostacyclin production more than platelet thromboxane formation in the rat. Prostaglandins 25: 693-709.

Sprecher, H. (1986) Comparison of omega-3 and omega-6 fatty acid metabolism. In: Health effects of polyunsaturated fatty acids in seafoods (Simopoulos, A.P., Kifer, R.R., and Martin, R.E. eds.), pp.353-379, Academic Press Inc., Orlando, FL.

Statistical Graphics Corporation (1988) STATGRAPHICS Users' Guide, Rockville, MD

Stubbs, C. D. & Smith, A. D. (1984) The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. Biochim. Biophy. Acta 779: 89-137.

Suematsu, T. & Abe, H. (1982) Liver and serum lipid peroxide levels in patients with liver diseases. In: Lipid peroxides in biology and medicine (Yagi, K. ed.), pp 285-293, Academic Press, NY

TenHoor, E. A., DeDeckers, M., Haddeman, E., Hornstra, G. & Quadt, J.F.A. (1980) Dietary manipulation of prostaglandin and thromboxane synthesis in heart , aorta, and blood platelets of the rat. In: Advances in Prostaglandin and Thromboxane Research. Vol. 8 (Samuelsson, B. ed.), pp. 1771-1778, Raven Press, New York

Thorngren, M. & Akesson, B. (1987) Effect of dietary fish on plasma selenium and its relation to haemostatic changes in healthy adults. Internat. J. Vit. Nutr. Res. 57: 429-435.

Thorngren, M., Gustafson, A. (1981) Effects of 11-week increase in dietary eicosapentaenoic acid on bleeding time, lipids, and platelet aggregation. Lancet ii: 1190-1193.

Thorngren, M., Shafi, S. & Born, G. V. R. (1984) Delay in primary haemostasis produced by a fish diet without change in local thromboxane A_2. Br. J. Haematology 58: 567-578.

Toivanen, J. L. (1987) Effects of selenium, vitamin E and vitamin C on human prostacyclin and thromboxane synthesis in vitro. Prostaglandins Leukotrienes and Medicine 26: 265-280.

Turk, J. (1980) Inactivation of vascular prostacyclin synthetase by platelet lipoxygenase products. Biochim. Biophys. Res. Commun. 95: 1628-1634.

U.S. Bureau of the Census (1988) Statistical abstract of the United States, 1988, 108th ed., pp.73-81, p.108, Washington DC

VasDas, F. W., Gibney, M. J.& Taylor, T. G. (1982) The effect of polyunsaturated fatty acids of the n-3 and n-6 series on platelet aggregation and platelet and aortic fatty acid composition in rabbits. Atherosclerosis 43: 245-247.

VonSchacky, C., Fischer, S. & Weber, P.C. (1985) Long-term effects of dietary marine w-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. J. Clin. Invest., 76: 1626-1631.

Wang, J., Lu, Y., Zhen, E., Guo, Z., Shi, F., and Liu, X. (1988) Effects of lipid peroxides on prostacyclin and thromboxane generation in hypercholesterolemic rabbits. Exp. Mol. Path. 48; 153-160.

Wang, Y. X. & Kiem, J. (1988) Effect of selenium supplementation on platelet selenium, glutathione peroxidase, and aggregation. Biol. Trace Elem. Res. 15: 89-96.

Warso, M. A. & Lands, W. E. M. (1983) Lipid peroxidation in relation to prostacyclin and thromboxane physiology and pathophysiology. Brit. Med. Bull. 39: 277-280.

Willms, P., Chanmugam, P. & Hwang, D.H. (1987) Vitamin E deficiency and prostaglandin synthesis in rat testes. Nutr. Res. 7:273-284.

Witting, L. A. (1967) Effect of antioxidant deficiency on tissue lipid composition in the rat. IV. Peroxidation

and interconversion of polyunsaturated fatty acids in muscle phospholipids. Lipids 2: 109-113.

Witting, L.A. & Horwitt, M.K. (1967) The effect of antioxidant deficiency on tissue lipid composition in the rat. I. gastricnemius and quadriceps muscle. Lipids 2: 89-96.

Witting, L.A., Theron, J. J. & Horwitt, M. K. (1967) The effect of antioxidant deficiency on tissue lipid composition in the rat. II. liver. Lipids 2: 97-102.

Wong, S. H., Nestel, P. J., Trimble, R. P., Storer, G. B., Illman, R. J. & Topping, D. L. (1984) The adaptive effects of dietary fish and safflower oil on lipid and lipoprotein metabolism in perfused rat liver. Biochem. Biophys. Acta. 792: 103-109.

Yokoyama, C., Mizuno, K., Mitachi, H., Yoshimoto, T., Yamanoto, S. & Pace-Asciak, C.R. (1983) Partial purification and characterization of arachidonate 12-lipoxygenase from rat lung . Biochim. Biophys. Acta 750: 237-243.

Yoshimoto, T., Yamamoto, s., Okuma, M.& Hayaishi, O. (1977) Solubilization and resolution of thromboxane synthesizing system from microsomes of bovine blood platelets. J. Biol. Chem. 252: 5871-5874.

Zmuda, A. Dembinska,-Kiec, A., Chykoski, A. & Gryglewski, R. J. (1977) Experimental atherosclerosis in rabbits: platelet aggregation, thromboxane A_2 generation and anti-aggregatory potency of prostacyclin. Prostaglandins 14: 1035-1042.