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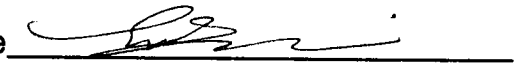
Signature 

Date Aug 1994

The Antithetic Relationship of Dietary Arachidonic Acid and Eicosapentaenoic Acid: A Comparison of Indices of Lipid Metabolism and Eicosanoid Production

A *Thesis*

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Bangyan Li

August, 1994

DEDICATION

This thesis is dedicated to my parents

Dr. Shan-Min Li

(李善民教授)

and

Dr. Jue-Ning Chen

(陳覺凝教授)

who have loved and supported me throughout my life, and made certain I had the opportunity to obtain an education.

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Abstract

Low to moderate levels of dietary arachidonic acid (20:4 n-6, AA) significantly enriches AA in tissue phospholipids and augment the *in vitro* production of its oxidized derivatives, eicosanoids. The effects of eicosapentaenoic acid (20:5 n-3, EPA) appear to be diametrically opposite to those of AA. This study is designed to evaluate this antithetic relationship of dietary AA and EPA. Forty-nine CD-1 mice were randomly assigned to four dietary groups: oleic acid (18:1 n-9, OA) group as control, AA group, EPA group, and AA+EPA group. Each diet contained identical amounts of saturated fat, monounsaturated fat, and polyunsaturated fat. Additionally, each diet was supplemented with combinations of OA, AA and EPA ethyl esters (3%, w/w). Animals were on diets for 4 weeks. Prior to sacrifice, production and release of eicosanoids were induced by stimulating residence peritoneal macrophages with opsonized zymosan. Fatty acid composition was analyzed in perfused liver. AA feeding significantly enriched AA concentration in hepatic phospholipids. Dietary EPA enriched hepatic phospholipids with EPA at the expense of AA. Adding AA to the EPA containing diet replenished tissue AA content to the control levels, while tissue EPA levels declined from 6.5 mole% in the EPA group to 0.4 mole % in the AA+EPA group. Similar effects were also observed with eicosanoid production. AA feeding significantly enhanced the formation of eicosanoids while dietary EPA depressed them. AA+EPA feeding elevated eicosanoid formation by 46% and 390% compared to the OA control group ($p<0.05$) and the EPA group ($p<0.05$), respectively. These changes of eicosanoid formation appeared to be

regulated in part by phospholipid AA content, as phospholipid AA content was significantly ($p<0.01$) and positively correlated with the formation of AA-derived eicosanoids. In summary, while dietary EPA potently depresses phospholipid AA content and AA-derived eicosanoid formation, dietary AA diminishes these effects of EPA and appears to be a more potent modulator for fatty acid composition and eicosanoid formation.

Preface

The Committee on Diet and Health (NRC) recommends that total consumption of fat should not exceed 30% of calories, and that 10% or less should be polyunsaturated fatty acids (PUFAs). PUFAs are important nutrients because they are not only essential, but they also possess the ability to decrease blood lipids, especially cholesterol. However, PUFAs must not be looked upon as a homogeneous group of fatty acids, but are composed of subgroups with different biological effects.

PUFAs can be divided into n-6 PUFAs and n-3 PUFAs. One of the most important n-3 PUFAs, eicosapentaenoic acid (EPA), is found primarily in fish products. EPA consumed as fish or fish oil had been known to decrease the risk of heart disease in Greenland Eskimos and Japanese fisherman. Arachidonic acid (AA), one of the more important n-6 PUFAs is found primarily in animal products. Recently, the levels of AA in the plasma has been linked to an increase in the risk of heart disease. The apparent difference in the risk of heart disease between AA and EPA is thought to be related in part to their abilities to modulate the production of a biologically active compounds, called eicosanoids. Eicosanoids are extensively involved in many biological functions such as blood clotting and inflammation. When excessively produced, these eicosanoids can be potentially harmful and could be a factor that contributes to heart disease.

AA is the major source for the formation of eicosanoids. Increasing dietary consumption of AA can elevate eicosanoid formation. Dietary EPA, on the other hand, inhibits the production of eicosanoids from AA. At the same time, dietary EPA produces its own eicosanoids, many of which are less biologically active or biologically inactive and thus are perceived to be beneficial. Therefore, manipulating AA and EPA consumption can change the level and type of eicosanoids produced in our body.

The research presented in this thesis was designed to evaluate whether dietary AA could diminish the beneficial effects of dietary EPA when both are included in the diet simultaneously. The results of this study demonstrated that when equal amounts of AA and EPA are consumed, AA is the more potent PUFA in modulating eicosanoid formation and thus, may be important in diminishing the beneficial effects associated with n-3 PUFAs.

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List of Abbreviations

5-lipoxygenase activating protein	FLAP
6-keto prostaglandin $F_{1\alpha}$	6-keto-$PGF_{1\alpha}$
12-hydroxyeicosatetraenoic acid(s)	12-HETE(s)
Arachidonic acid	AA
Cyclic adenosine 3', 5' monophosphate	cAMP
Cyclic guanosine 3', 5' monophosphate	cGMP
Docosahexaenoic acid	DHA
Eicosapentaenoic acid	EPA
Gas chromatography	GC
High performance liquid chromatography	HPLC
Leukotriene A_4	LTA_4
Leukotriene B_4	LTB_4
Leukotriene B_5	LTB_5
Leukotriene C_4	LTC_4
Leukotriene C_5	LTC_5
Leukotriene D_4	LTD_4
Leukotriene D_5	LTD_5
Leukotriene E_4	LTE_4
Leukotriene E_5	LTE_5
Leukotriene(s)	LT(s)
Linoleic acid	LA

Linolenic acid	LNA
Lipoxygenase	LOX
Oleic acid	OA
Polyunsaturated fatty acid(s)	PUFA(s)
Prostacyclin I_2	PGI_2
Prostacyclin I_3	PGI_3
Prostacyclin(s)	PGI(s)
Prostaglandin B_1	PGB_1
Prostaglandin E_1	PGE_1
Prostaglandin E_2	PGE_2
Prostaglandin E_3	PGE_3
Prostaglandin $F_{1\alpha}$	$PGF_{1\alpha}$
Prostaglandin $F_{1\beta}$	$PGF_{1\beta}$
Prostaglandin $F_{2\alpha}$	$PGF_{2\alpha}$
Prostaglandin $F_{3\alpha}$	$PGF_{3\alpha}$
Prostaglandin H_2	PGH_2
Prostaglandin H(s)	PGH(s)
Prostaglandin H synthetase	PGHS
Prostaglandin(s)	PG(s)
Radioimminoassay	RIA
Tertiary butylhydroquinone	TBHQ
Thin layer chromatography	TLC
Thromboxane A_2	TXA_2
Thromboxane A_3	TXA_3
Thromboxane(s)	TX(s)

Chapter I
Literature Review

Dietary fats contain important essential nutrients that are required to maintain human health. They facilitate the absorption of fat soluble vitamins, are important sources of energy, and are essential in maintaining membrane integrity. Dietary fats are composed of a glycerol backbone and an array of fatty acids associated as ester linkages. Fatty acids can be categorized into three major groups: saturated fatty acids, which have no double bonds, monounsaturated fatty acids, which have only one double bond and polyunsaturated fatty acids (PUFAs), which have more than one double bond. Essentiality of the fatty acids are provided by the PUFAs.

I. Historical perspective of the essential fatty acids and eicosanoids

About 60 years ago, two seemingly unrelated developments occurred. One led to the discovery of essential fatty acids; the other to the discovery of prostaglandins and other related compounds, generally classified as eicosanoids.

Discovery of the essential fatty acids: The essentiality of fatty acids was first discovered by Burr and Burr in 1929 (Burr & Burr 1929). It was observed that animals consuming fat free diets developed a deficiency disease. Scaly paws and tails, growth retardation, fatty liver, kidney damage, reduced fertility, and high skin permeability were symptoms of this disease.

One year later, linoleic acid (18:2 n-6, LA), which is relatively rich in plant sources, was identified to be the substance required to prevent essential fatty acid deficiency (Burr & Burr 1930). Since then, the essential fatty acid deficiency syndrome has been observed in many species including humans (Table 1.1). Many fatty acids which are related structurally to LA, such as arachidonic acid (20:4 n-6, AA), gamma linolenic acid (18:3 n-6), and alpha linolenic acid (18:3 n-3, LNA) were found to prevent various aspects of essential fatty acid deficiency symptoms (Mead & Willis 1987).

Discovery of the prostaglandins: During the same period of time, Kurzrok and Lieb (1930) reported that human semen could cause either relaxation or constriction of the uterine muscle. In 1936, von Euler isolated a substance from homogenates of the prostate gland, which produced a similar response. This substance was named prostaglandin (PG) (von Euler 1936) because it was first isolated in the prostate gland. It was not until two decades later that a prostaglandin was finally purified. In the late 1950s and early 1960s, Bergström and Sjövall reported the purification of the crystalline forms of two structurally related PGs (Bergström and Sjövall 1957). Using the newly developed mass spectrum technique, the chemical formula of these crystals was elucidated to be unsaturated carboxylic acids with 20 carbon chain length and no nitrogen atoms (Bergström and Sjövall 1960). These two purified compounds were then named prostaglandin E_1 (PGE_1) and prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$). Later, it was determined that the reduction products of PGE_1 are two epimers, $PGF_{1\alpha}$ and $PGF_{1\beta}$ (Bergström *et al.* 1963). The structures of many other similar compounds were soon

Table 1.1 Differing characteristics of n-3 and n-6 essential fatty acid deficiencies[1]

	N-6 PUFAs	N-3 PUFAs
Clinical features	Growth retardation	Normal skin, growth and reproduction
	Skin lesions	Reduced learning
	Reproductive failure	Abnormal electroreinogram
	Fatty aliver	Impaired vision
	Polydipsia	Polydipsia
Biochemical markers	Decreased 18:3 n-3 and 22:6 n-3	Decreased 18:2 n-6 and 20:4 n-6
	Increased 22:4 n-6 and 22:5 n-6	Increased 20:3 n-9 (only if n-3 also low)
	Increased 20:3 n-9 (only if n-6 also low)	

[1]see Connor *et al.* 1992, Salem & Ward 1993, Carlson & Salem 1991, and Okuyama 1992.

elucidated, including the 2-series PGE_2 and $PGF_{2\alpha}$, and the 3-series PGE_3 and $PGF_{3\alpha}$ (Samuelsson 1970).

The structures of these early prostaglandins suggested that their precursor was probably a 20 carbon PUFA with methylene interrupted double bonds. However, it was not until radiolabeled AA was incubated with homogenates of sheep vesicle gland that PUFA was identified as the metabolic precursor (van Dorp 1964). Subsequently, the PUFA origins were found for the 1- series and the 3- series prostaglandins (Fisher & Weber 1983 & 1984, and Klenberg & Samuelsson 1965). van Dorp suggested that AA is the main source of prostaglandin synthesis due to its strong biological effects compared to the other two prostaglandin precursors, eicosatrienoic acid and eicosapentaenoic acid (van Dorp 1964). Soon after, a prostaglandin synthetic pathway was proposed, which involved the enzyme catalyzed incorporation of two molecules of oxygen into a PUFA with methylene interrupted double bonds (Nugteren *et al.* 1966).

These discoveries eventually led to the brilliant work of Hamberg and Samuelsson *et al.* in the late 1960s and 1970s. They discovered the mechanisms of PGE_1 and $PGF_{1\alpha}$ biosynthesis (Hamberg & Samuelsson 1967a), and the enzyme responsible for PG biosynthesis, prostaglandin H synthase (PGHS) (Hamberg & Samuelsson 1973, and Hamberg *et al.* 1974). The discovery of prostaglandin intermediates, prostaglandin H (PGH) (Hamberg & Samuelsson 1973) later led to the discovery of several new compounds derived from PGH_2, *i. e.* thromboxane A_2 (TXA_2) which induces

platelet aggregation (Hamberg *et al.* 1975) and prostacyclin I_2 (PGI_2) which inhibits platelet aggregation (Moncada *et al.* 1976).

At the same time, leukotrienes were discovered by Hamberg and Samuelsson. They reported that compounds other than prostaglandins were formed following the metabolism of AA and suggested a second pathway independent of the prostaglandin pathway (Hamberg & Samuelsson 1967b & 1974). These compounds were later named leukotrienes (LTs) as they were isolated from the leukocytes and contain a conjugated triene structure (Borgeat *et al.* 1976, Borgeat & Samuelsson 1979, and Samuelsson *et al.* 1979). The enzymes, lipid oxygenases which catalyzed the formation of leukotrienes and other related compounds were called lipoxygenases (LOX) (Hamberg & Samuelsson 1974, Borgeat & Samuelsson 1979, and Samuelsson *et al.* 1979).

Since then, many other compounds structurally related to prostaglandins and leukotrienes have been discovered and added to the metabolic pathway of AA, generally referred to as the AA cascade. These compounds are called eicosanoids, referring to the oxidized derivatives of the 20 carbon PUFAs.

It is now readily accepted that the effects of the essential fatty acids and eicosanoids are related, as impairment of eicosanoid formation may contribute to the symptoms observed with essential fatty acid deficiency. The biological functions of the eicosanoids will be discussed later in this review.

II. Metabolism of polyunsaturated fatty acids and eicosanoids

Metabolism of PUFAs: There are two major PUFA families: n-6 and n-3 PUFA. They are named after the position of the first double bond from the methyl terminal of the fatty acid (Fig 1.1). The n-6 PUFA family includes the essential fatty acid, LA, which can be metabolized to longer chain highly unsaturated n-6 PUFA, such as AA. LA is found primarily in vegetable oils, while AA is found primarily in animal products. The metabolic precursor of the n-3 PUFA family is LNA which is found primarily in vegetable products, especially vegetable oils. LNA can be metabolized to form eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), biologically important fatty acids found in fish oils (Fig 1.1). Fatty acids from different PUFA families are not interconvertable (Mohrhauer & Holman 1963c).

LA and LNA are similarly metabolized (Fig 1.1). They are desaturated and elongated to C_{20-24} fatty acids containing up to 6 double bonds. The $\Delta 5$ and $\Delta 6$ desaturases are important regulatory steps in the pathways, with $\Delta 6$ desaturase being the rate limiting enzyme (Brenner *et al.* 1977, Nugteren 1962). Importantly, LA, LNA and their derivatives compete as substrates for the same enzymes in the metabolic pathways (Brenner and Peluffo 1969).

Further conversions of AA and EPA to the longer chain fatty acids were logically hypothesized for thirty years to be catalyzed by a $\Delta 4$ desaturase. However, recent studies have suggested that the existence of

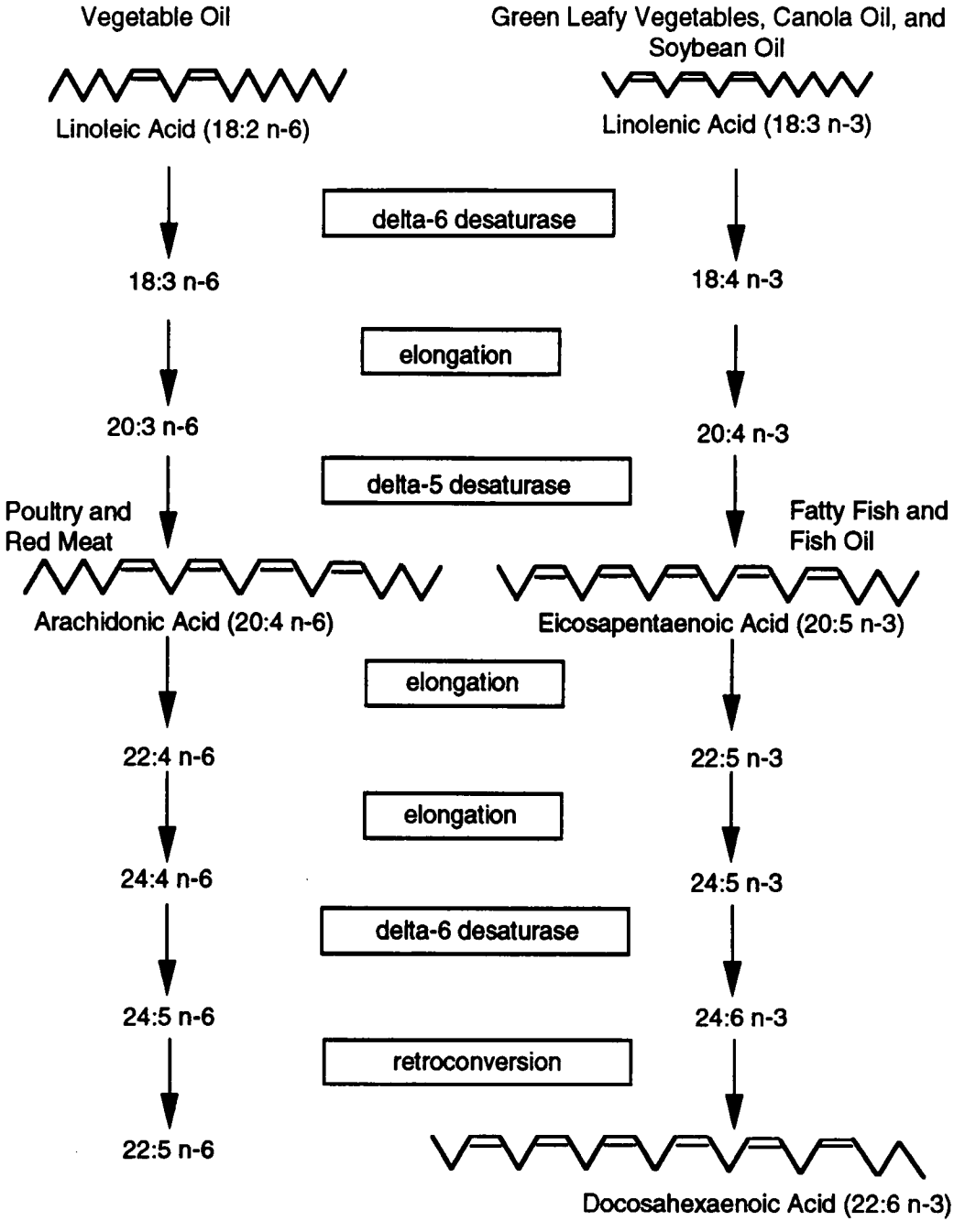

Fig 1.1 Metabolisms of the n-6 and n-3 PUFAs. Fatty acids from each PUFA family are converted to each other *via* desaturation and elongation with delta-6 desaturase being the rate limiting enzyme. Fatty acids from different PUFA families cannot be converted to each other. See Brenner *et. al.* 1969, Brenner 1977, and Voss *et al.* 1991.

this enzyme is in doubt (Voss *et al.* 1991). Ayala and coworkers failed to detect any Δ4 desaturase activity in testes even though it contains large amounts of 22:5 n-6, the apparent desaturation and elongation product of AA (Ayala *et al.* 1973, and Carpenter 1971). A new pathway was later suggested in 1991 by Voss *et al.*. They suggested that AA and EPA were first elongated to the 24 carbon products followed by desaturation by a putative Δ6 desaturase and then retroconverted to 22 carbon products through a chain shortening reaction referred to as retroconversion (Fig 1.1). Desaturation and elongation occur in the endoplasmic reticulum, while the peroxisomes appear to be the site of retroconversion (Rosenthal *et al.* 1991, Hiltunen *et al.* 1986). Thus, it is suggested that the 24 carbon fatty acids are formed in the endoplasmic reticulum and then transported to the peroxisomes for further processing into the 22 carbon products (Voss *et al.* 1991).

Further studies by the same group attempted to determine whether there is chain-length-specific Δ6 desaturase (Geiger *et al.* 1993). Their results demonstrated that the 18 carbon and 24 carbon Δ6 desaturase substrates compete with each other for the enzyme, suggesting that these Δ6 desaturases may be one in the same. However, the question whether multiple forms of Δ6 desaturase exist still remains to be determined.

After elongation and desaturation, PUFAs and their metabolic products are incorporated primarily into phospholipids at the C-2 position by the action of acyltransferases (Brenner & Nervi 1965, Lands *et al.* 1982,

McKean *et al.* 1982). Of importance are the 20 carbon PUFAs, AA and EPA, which are precursors for eicosanoids. AA and EPA can be released from phospholipids upon cell stimulation and be enzymatically converted to their oxidative derivatives, PGs and LTs.

Metabolism of eicosanoids: Eicosanoids are produced via three pathways: the prostaglandin H synthase (PGHS) pathway which is responsible for the formation of prostaglandins (PGs), thromboxanes (TXs) and prostacyclins (PGIs); the lipoxygenase (LOX) pathway which generates leukotrienes (LTs); and the cytochrome P450 enzyme system (Capdecvila *et al.* 1992) which generates many hydroxy and epoxy compounds from the fatty acid precursors. The first two pathways are studied most often and their products will be used as measurable parameters in this study (Fig 1.2).

PGH synthase pathway: PGHS introduces oxygen atoms into AA, resulting in the characteristic endoperoxide ring. The product of PGHS is the intermediate PGH_2, which then is further reduced to form PGE_2 (Egan *et al.* 1979). Thromboxane synthase and prostacyclin synthase convert PGH_2 into TXA_2 and PGI_2 respectively (Moncada *et al.* 1976, and Hammarström & Falardeau 1977). Two isoforms of PGHS have been identified (Hemler *et al.* 1976, O'Banion *et al.* 1991, and Fletcher *et al.* 1992). PGHS I is responsible for the constitutive production of PGs in cells and PGHS II, the inducible form of the enzyme, is responsible for the elevated production of PGs during inflammation. The crystal structure of PGHS I clearly demonstrates the double functionality of the enzyme (Picot *et al.* 1994). The two separated

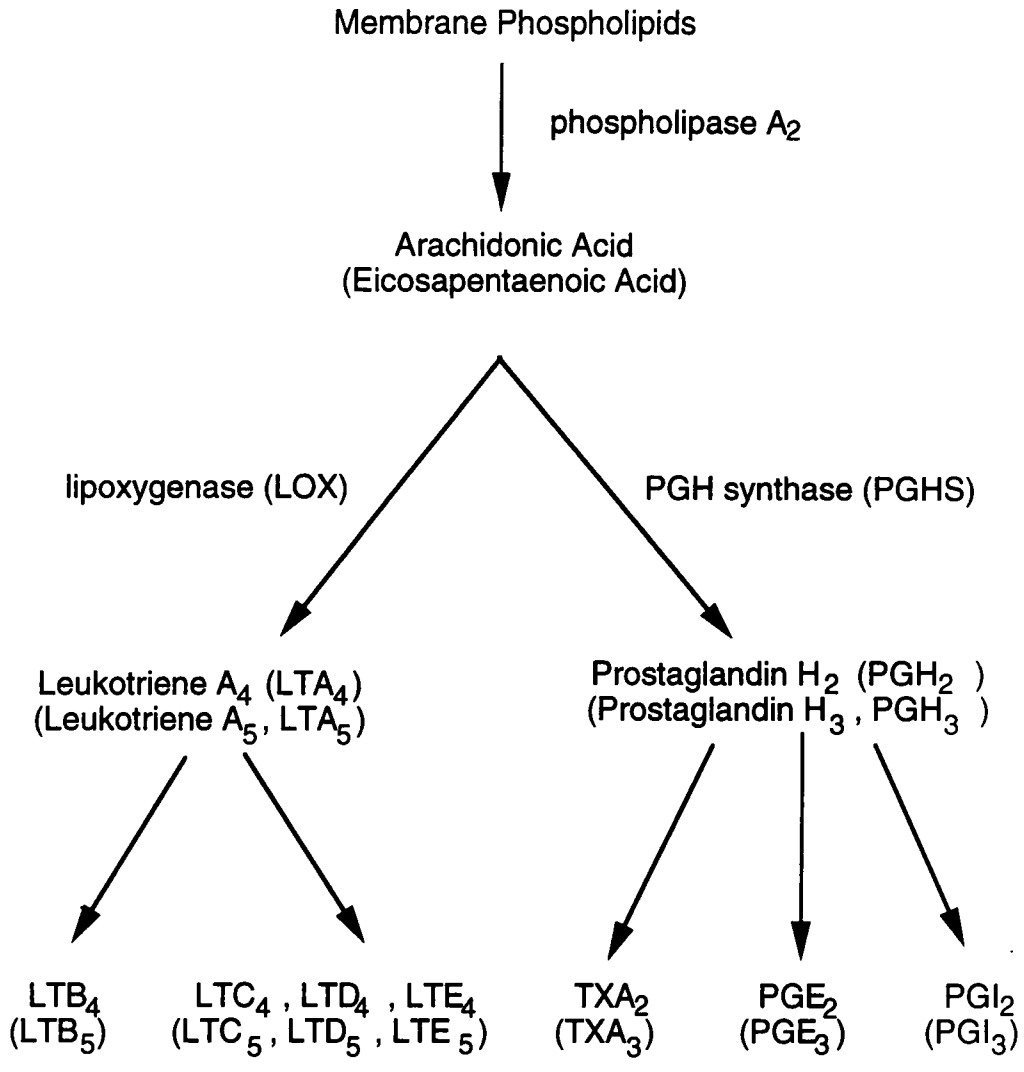

Fig 1.2 Lipoxygenase and PGH synthase pathway. Arachidonic acid is released from membrane phospholipids and converted to leukotrienes and prostanoids through lipoxygenase and PGH synthase pathways. LTB_4, leukotriene B_4, LTB_5, leukotriene B_5 LTC_4, leukotriene C_4; LTC_5, leukotriene C_5; LTD_4, leukotriene D_4; LTD_5, leukotriene D_5; LTE_4, leukotriene E_4, LTE_5, leukotriene E_5; TXA_2, thromboxane A_2; PGE_2, prostaglandin E_2 PGI_2, prostacyclin I_2. TXA_3, thromboxane A_3; PGE_3, prostaglandin E_3; PGI_3, prostacyclin I_3, See van Drop *et al.* 1964, Borgeat & Samuelsson 1979, Hamberg & Samuelsson 1973 & 1974, Hamberg *et al.* 1975.

domains, peroxidase domain and cyclooxygenase domain, explain the biological observation that PGHS has both peroxidase activity and cyclooxygenase activity (Hemler *et al.* 1976, and Hemler & Lands 1980). PGHS I is an integral membrane protein located primarily in the endoplasmic reticulum (Smith & Marnett 1991), while the exact location of PGHS II is not clear, but is also suspected to be associated with the outer membrane of the endoplasmic reticulum.

Lipoxygenase pathway: LT synthesis is catalyzed by LOX enzymes, the most importance of which is the 5-LOX (Yamamoto 1992). The immediate product of the 5-LOX is a hydroperoxide derivative of AA, which then is converted to the 5, 6 epoxide, leukotriene A_4 (LTA_4), by LTA_4 synthase. The 5-LOX contains both 5-LOX and LTA_4 synthase activities. Leukotriene B_4 (LTB_4) is a dihydroxy product of LTA_4, while leukotriene C_4 (LTC_4), leukotriene D_4 (LTD_4), leuktriene E_4 (LTE_4) are glutathionyl, cysteinylglycyl, and cysteinyl derivatives of LTA_4, respectively. 5-LOX was found to be translocated to the nuclear membrane upon cellular stimulation (Kargman *et al.* 1991, and Rouzer & Kargman *et al.* 1988). Here, AA is released from membrane phospholipids by phospholipase A_2 and presented to 5-LOX by a 5-lipoxygenase activating protein, commonly referred to as "FLAP", associated with the nuclear membrane (Peters-Golden & McNish 1993).

The biological effects of eicosanoids: Eicosanoids appear to be ubiquitously produced. Earlier studies focused on their biological effects in

the vascular system. TXA_2, the primary eicosanoid produced in platelets, is a potent proaggregant and vasoconstrictor, while PGI_2 has the opposite effects on vascular and platelet aggregation (Moncada & Vane 1979). PGE_2 is a primary prostaglandin produced by leukocytes and is particularly important for lymphocytes and macrophage functions (Table 1.2). PGE_2 is a potent immunosuppressant at high levels (10^{-8} M), but, at low levels (10^{-10} M), stimulates T lymphocytes maturation (Shapiro *et al.* 1993, Grbic *et al.* 1991, Stone *et al.* 1990). LTB_4 is a potent chemotactic and chemokinetic agent produced primarily by leukocytes that stimulates the migration and mobility of leukocytes (Table 1.2), and the release of lysozymes and histamine (Molski *et al.* 1981,Snyderman & Goetzl 1981, Krauss *et al.* 1994, Spada *et al.* 1994). LTB_4 also inhibits the activity of T helper cells and enhances T suppresser cells and natural killer cells (Goodwin & Behrens 1988).

Unlike LTB_4, the sulfidopeptide leukotrienes, LTC_4, D_4, and E_4, induce broncho and gastrointestinal tract smooth muscle contraction with LTE_4 having the least biological activity of the three (Fedldberg & Kellaway 1938, and Kellaway and Trethewie 1940). The bronchoconstriction effects of sulfidopeptide leukotrienes can be as much as 1000 times more potent than that of histamine (Christie *et al.* 1993). They are also transient vasoconstrictors which induce transient arteriolar contractions followed by plasma leakage (Dahlén *et al.* 1981).

Table 1.2 Some Examples of Biological Functions of Eicosanoids[1]

Eicosanoid Compound	Primary Cells	Biological Effects
PGE_2	Leukocytes	Weak vasodilation, Immunoregulatory, Bronchodilation
PGE_3	Leukocytes	Weak vasodilation (less potent than PGE_2)
PGF_{2a}	Leukocytes	Vasoconstriction
TXA_2	Platelete, Macrophages	Vasoconstriction, platelet adhesion and aggregation
TXA_3	Platelete, Macrophages	Biological inactive
PGI_2	Endothelial cells, kidney lung, Macrophages	Antiaggregatory effects, bronchodilation
PGI_3	Same as above	Biological activity is the same as PGI_2
LTB_4 and LTB_5	Leukocytes	Leukocytes adhesion and chemotaxis, Lysozyme and histamine release, release of reactive oxygen species
LTC_4, LTD_4, LTE_4	Leukocytes	Bronchoconstriction, vasoconstriction and vascular permeability
LTC_5, LTD_5, LTE5	Leukocytes	same as above (might be less biologically potent than the 4-series)

[1] PGA_2, prostaglandin A_2; PGE_2, prostaglandin E_2; PGE_3, prostaglandin E_3; PGF_{2a}, prostaglandin F_{2a}; TXA_2, thromboxane A_2; TXA_3, thromboxane A_3; PGI_2, prostacyclin I_2; PGI_3, prostacyclin I_3; LTB_4, leukotriene B_4; LTB_5, leukotriene B_5; LTC_4, leukotriene C_4, LTC_5, leukotriene C_5; LTD_4, leukotriene D_4; LTD_5, leukotriene D_5; LTE_4, leukotriene E_4; LTE_5, leukotriene E_5.

The biological effects of the EPA-derived eicosanoids, namely the 3-series of PGs, PGIs, TXs and 5-series LTs, are similar to those of the AA-derived eicosanoids. However, they tend to be less biologically potent (Fisher & Weber 1983, Chang *et al.* 1989, Tatsuno *et al.* 1990), with the exception of PGI_3 (Fishcer & Weber 1984) and possibly the sulfidopeptide leukotrienes as the relative biological potency of the 5-series sulfidopeptide leukotrienes is currently being debated (Dáhlen *et al.* 1981, Wallace & McKnight 1990).

At the cellular level, the biological effects of these eicosanoids appear to be acting through the cyclic nucleotides, such as adenosine-3', 5'-monophosphate (cAMP) and guanosine-3',5'-monophosphate (cGMP) (Butcher & Baird 1968, Dunham *et al.* 1974). PGE_2 elevates cAMP levels by stimulating adenylate cyclase (Gorman 1975). In platelet, TXA_2 decreases and PGI_2 increases cAMP levels (Gorman 1979). LTs also exert their effects through the cyclic nucleotides (Molski *et al.* 1981).

III. Regulation of PUFA and eicosanoid metabolism

Eicosanoids are potent proinflammatory agents involved in cell signaling. Therefore, it is of relevance to regulate the production of these compounds. Formation of eicosanoids derived from AA and EPA depends largely upon the tissue levels of AA and EPA (Kinsella *et al.* 1990a). Phospholipid AA and EPA levels can be altered by exogenous lipids, such as dietary fatty acids, particularly LA, AA and EPA.

Regulation of eicosanoid formation by dietary means: Dietary LA not only elevates tissue levels of LA in membrane phospholipids but also increases levels of its metabolic product, AA (Fig 1.3). However, the ability of LA in enriching tissue AA content is limited due to product feedback inhibition of AA on the rate limiting enzyme, Δ6 desaturase (Nervi *et al.* 1968, and Brenner *et al.* 1969). Increasing the consumption of LA appears to elevate tissue content of AA in a linear manner up to 2% of calorie (2 en%) (Whelan *et al.* 1992, James *et al.* 1993, Emken 1988). However, at higher levels, LA appears to have no additional effects on tissue AA content (Mohrhauer & Holman 1963a, James *et al.* 1993).

Dietary AA, on the other hand, by-passes these regulatory steps (*i.e.* Δ6 and Δ5 desaturase), and enriches tissue phospholipids with AA. Subsequently, the formation of AA-derived eicosanoids can be enhanced (Fig 1.4). Increasing amounts of dietary AA results in the elevation of AA in tissue phospholipids at the expense of LA and n-3 PUFAs (Danon *et al.* 1975, Mohrhauer & Holman 1963b, Whelan *et al.* 1992 & 1993). As a result, AA-derived eicosanoid formation is subsequently increased. A pioneering study with dietary AA was reported in 1975 by Seyberth *et al.* (Seyberth *et al.* 1975). In this study, four human subjects were supplemented with 6 g AA ethyl ester for 2 weeks. Systemic prostaglandin production, as measured by the urinary PGE_2 metabolite, 7α-hydroxy-5, 11-diketotetranorprostane-1, 16-dioic acid, was elevated during AA feeding and returned to control levels after AA was removed from the diet. In hamsters, low to moderate levels of dietary AA (1.1%, w/w) were shown to enhance the production of many AA-

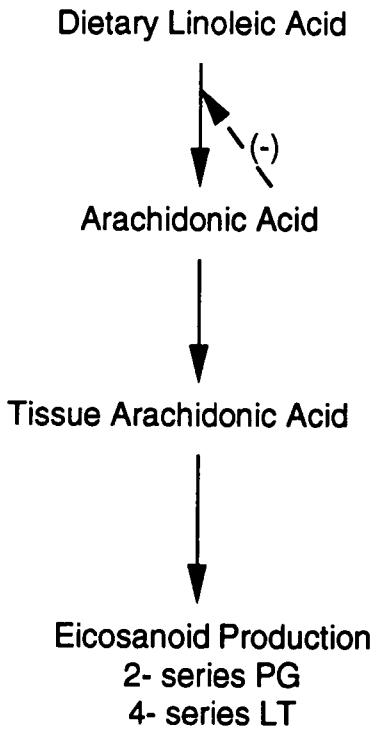

Fig 1.3 Regulation of tissue arachidonic acid (AA) and AA-derived eicosanoid production by dietary linoleic acid (LA). Upon consumption, LA is converted to AA, and tissue AA is the major precursor for eicosanoid production. However, this conversion is highly regulated through production feedback inhibition. Therefore, the ability of LA in regulating tissue AA and AA-derived eicosanoid production is limited. PG, prostanoids; LT, leukotrienes. Solid lines represent normal metabolic pathway; dotted lines show inhibition or negative effect. See Whelan *et al.* 1993.

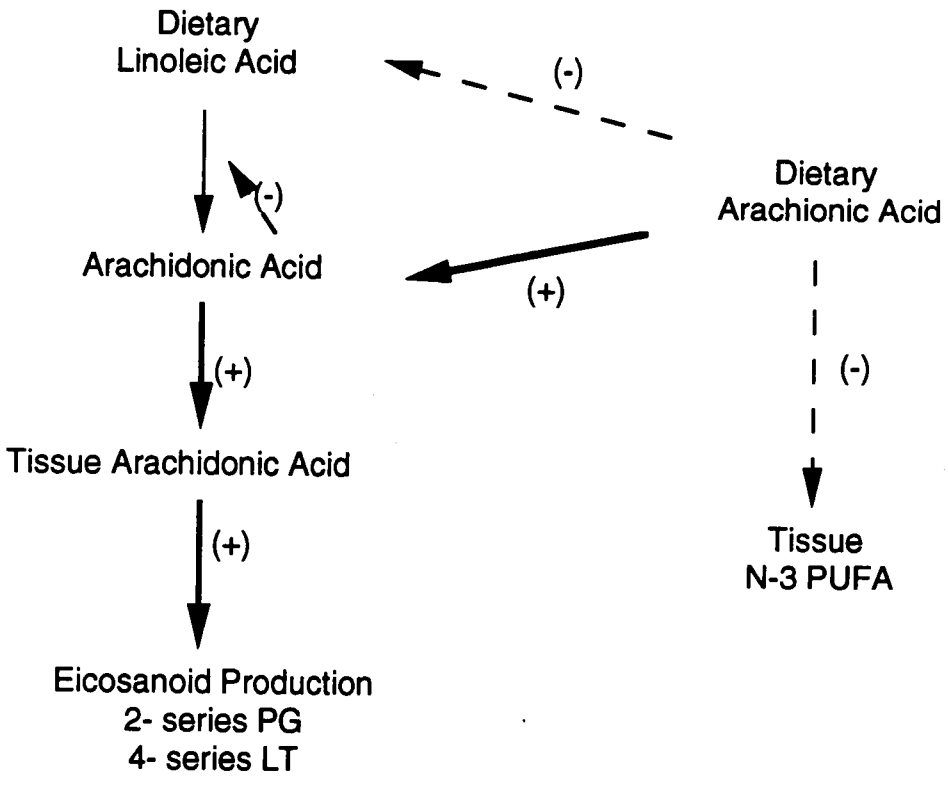

Fig 1.4 Regulation of tissue arachidonic acid (AA) and eicosanoid production by dietary AA. Dietary AA enriches tissue phospholipids with AA, and upon cell stimulation, augments eicosanoid formation. PG, prostanoids; LT, leukotrienes. Solid lines represent normal metabolic pathway; dotted lines show inhibition or negative effect; and bold solid lines show enhancement of pathways or a positive effect. See Whelan *et al.* 1993.

derived eicosanoids, including PGE_2, TXA_2, LTC_4, LTE_4, and 12-hydroecosatetraenoic acids (12-HETES), in isolated platelets and macrophage stimulated *in vitro* (Whelan *et al.* 1993). PGI_2 production was not shown to be elevated in this study; however, it was later shown to be enhanced by AA feeding by others (Mann *et al.* 1994). Therefore, dietary AA is a potential regulator for the production of AA-derived eicosanoids (Fig 1.4).

The effects of dietary n-3 PUFAs are in opposition to those observed with n-6 PUFAs. Dietary LNA enriches tissue LNA and tissue levels of its metabolic products EPA and DHA (Boudreau *et al.* 1991, and Mohrhauer & Holman 1963b, Hwang *et al.* 1988, Whelan *et al.* 1991). As in the case of LA, the enrichment of the metabolic products of LNA is limited by feedback inhibition (Whelan *et al.* 1991, Boudreau *et al.* 1991, Hwang *et al.* 1988, Sanders & Hochland 1983).

Intake of long chain n-3 PUFAs elevate their tissue levels in membrane phospholipids and enhance EPA-derived eicosanoid formation (Fig 1.5). Consuming longer chain n-3 PUFAs, such as EPA, enrich tissues with n-3 PUFA and EPA at the expense of AA (Whelan *et al.* 1993, Lee *et al.* 1992, and Broughton *et al.* 1991). Subsequently, EPA-derived eicosanoid formation is enhanced while AA-derived eicosanoid formation is suppressed (Hwang *et al.* 1988, Broughton *et al.* 1991, Whelan *et al.* 1991 & 1993, Mann *et al.* 1994)

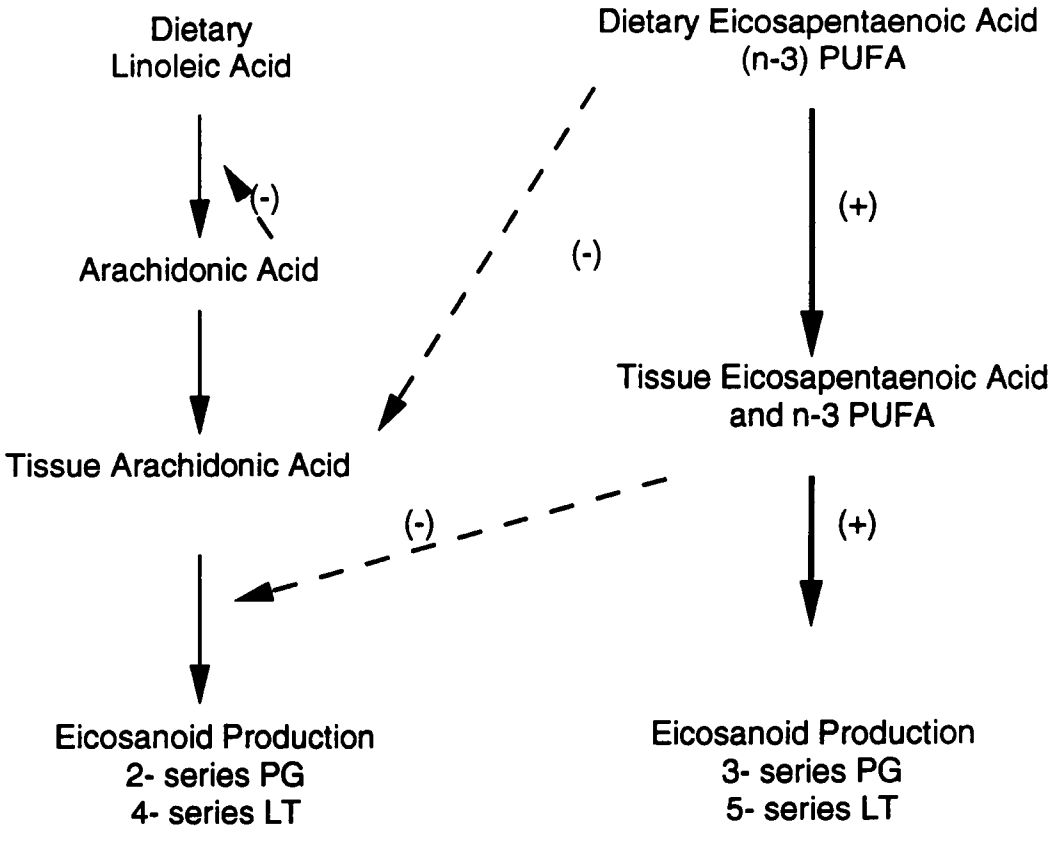

Fig 1.5 Dietary eicosapentaenoic acid (EPA) inhibits the incorporation of arachidonic acid (AA) into tissue phospholipids and suppresses the production of AA-derived eicosanoid formation. When EPA is included in the diet, in the absence of AA, it increases tissue n-3 PUFA mainly at the cost of tissue AA. Also, EPA augments the production of EPA-derived eicosanoid formation and decreases the formation of AA-derived eicosanoids. PUFA, polyunsaturated fatty acids; PG, prostanoids; LT, leukotrienes; Solid lines represent normal metabolic pathway; dotted lines show inhibition or negative effect; and bold solid lines show enhancement of pathways or a positive effect. See Whelan *et al.* 1993.

Competitions of AA and EPA for tissue incorporation and eicosanoid formation: AA and EPA are structurally similar compounds which compete for enrichment in tissue phospholipids and the formation of eicosanoids. First, AA and EPA compete for elongation and desaturation. The n-3 fatty acids are preferred substrates for the Δ6 desaturase compared to the n-6 fatty acids (Geiger *et al.* 1993, Brenner & Peluffo 1969). Elongation and desaturation of AA to 22:5 (n-6) appears to be lower compared to the conversion of EPA to DHA, while the retroconversion of 22:5n-6 to AA is favored compared to the retroconversion of DHA to EPA in the rodent model (Geiger *et al.* 1993). Second, AA and EPA compete for incorporation into phospholipids. The acyltransferase (Fernández *et al.* 1992, Banerjee & Rosenthal 1985) have a predominant selectivity for AA (Iritani *et al.* 1984, Iritani and Narita 1984). The acyl transfer rate is higher when AA CoA is provided as the acyl donor compared to EPA CoA (Iritani *et al.* 1987). Also, depletion of AA from tissue phospholipids is more difficult than that of EPA upon removal of fatty acids from the diet. When animals were switched from a fat rich diet to a fat free diet, the rate of decrease in AA content in phospholipids was less than that of EPA (Iritani *et al.* 1987). As the result, AA and DHA are preferentially accumulated in tissue phospholipids. Therefore, AA is preferentially incorporated into membrane phospholipids compared to EPA. Third, AA and EPA compete as substrates for PGHS or LOX. EPA is a poor substrate for PGHS compared to AA (Culp *et al.* 1979); however, EPA can serve as an effective inhibitor of PGHS for the production of AA-derived PGs, TXs and PGIs. Substrate specificity of LOX appear to be

slightly higher for EPA than AA (Aharony & Stein 1986, and Ochi *et al.* 1982). However, it is unclear whether LOX has a substrate preference.

V. Scope and rationale of the study

The epidemiological studies pioneered by Bang and Dyerberg in the late 1970s led to an increased interest in the biological effects of fish oils (Dyerberg *et al.* 1975, Bang *et al.* 1980). Their research suggested that the consumption of n-3 PUFA may be related to the lower cardiovascular disease mortality rates associate with Greenland Eskimos compared to Danish controls. The conclusions of these studies were supported by data demonstrating that the consumption of n-3 PUFA, in the form of fish oil, inhibited the development of atherosclerotic lesions in Rhesus monkeys (Davis *et al.* 1987). These beneficial effects of n-3 PUFAs are believed to be related in part to their ability to effectively suppress the production of eicosanoids from AA (Carry *et al.* 1992, Ross 1987, Hajjar & Pomerantz 1992, Clark 1992, Ulbright & Southgate 1991).

Eicosanoids produced from AA are highly potent compounds that can be proinflammatory, proatherogenic and prothrombotic when produced excessively (Kinsella 1990). Therefore, it has been of concern that high intakes of LA might be potentially harmful since LA can contribute to eicosanoid formation through its conversion to AA (Kinsella & Lokesh 1990, Knapp 1988, Kinsella *et al.* 1990b). Fortunately, the conversion from LA to AA is highly regulated through product feedback inhibition (Brenner 1977).

However, AA consumed in the diet by-passes the regulatory steps involved in the conversion of LA to AA, enriching tissue phospholipids with AA, result in increased eicosanoid formation (Whelan *et al.* 1992 & 1993).

While AA and EPA are both potent regulators of eicosanoid formation, it has yet to be determined whether AA would diminish the effects of EPA when it is included in the diet with EPA. In 1982, a study by O'Dea and Sinclair reported that the plasma AA content of subjects fed fish increased unexpectedly (O'Dea & Sinclair 1982). Analysis of the fish consumed in this study showed high content of AA as well as n-3 PUFAs. This study was the first to indicate that dietary AA may abrogate some of the effects of n-3 PUFA. A more recent study also suggested that AA, when compared to EPA, is preferentially incorporated into tissue phospholipids within 4 hours of oral administration (Nilsson *et al.* 1993). Therefore, the current study is designed to directly and systematically evaluate the divergent effects of dietary AA and EPA. More specifically, *it intends to evaluate the antithetic relationship of dietary AA and EPA on lipid metabolism and eicosanoid formation .*

Objectives: Dietary AA significantly enhances the formation of AA-derived eicosanoids, while dietary EPA efficiently suppresses their formation and enhances the formation of EPA-derived eicosanoids. These effects on eicosanoid production are mediated in part by the abilities of the two PUFAs to alter phospholipid fatty acid composition. Therefore, three major objectives are considered in this work.

I. To determine the effect of dietary AA on AA-derived eicosanoid formation *in vivo*, *viz.* PGE_2, TXB_2, PGI_2, LTB_4 and LTE_4.

II. To determine whether including AA in a diet containing EPA will replenish tissue AA content which is depressed by dietary EPA.

III. To evaluate whether including AA in the diet will increase the production of AA-derived eicosanoids which are diminished by dietary EPA.

Hypothesis: Including equal amounts of AA and EPA in the diet may diminish the effects of EPA on tissue AA content and AA-derived eicosanoid formation.

Chapter II
Material and Method

I. Animals and diets

Animals: Forty-nine CD-1 male mice (Harlan Sprague Dawley, Indianapolis, IN) (21-24 g) were upon arrival, randomly assigned to 4 dietary groups, twelve or thirteen animals per group. All animals were maintained on chow diet for 1 week prior to being placed on experimental diets for 28 days. Animals were kept in a 12 hour dark/light cycle room with a temperature controlled between 60 and 70 °F.

A mouse model was chosen as the *in vivo* experimental model for this study. It has been used previously for the study of eicosanoid metabolism and lipid metabolism *in vivo* and *in vitro* (Whelan *et al.* 1991 & 1993). The reasons this animal model is chosen for this study are as follows:

I. Cell function is not always equivalent under *in vitro* and *in vivo* conditions. Therefore, the effects of dietary AA and EPA will be investigated under a more physiologically relevant condition (*in vivo*).

II. The use of human subjects would be ideal. However, the data which can be collected from human subjects is limited for obvious reasons.

III. Macrophages have similar roles in all mammals and have both PGHS and LOX enzymes, and thus the ability to produce PGs, TXs, PGIs and LTs.

IV. Eicosanoid production *in vivo* is well characterized in this mouse model (Whelan *et al.* 1991).

V. The mouse was used due to economic reasons. Using larger rodents, such as rat or hamster, were cost prohibitive.

VI. Mice are small animals, and are relatively easy to handle.

Diets: All diets contained 90% (w/w) fat free-mouse chow diet (Dyets Inc., Bethlehem, PA) and 10% (w/w) fat (Tables 2.1 and 2.2). The LA content of the diets, as provided primarily by safflower oil (75% LA)(Hollywood, St. Louis, MO), was maintained at 1.8% (w/w). Each diet also contained olive oil (1.5%, w/w) (Kroger, Cincinnati, OH) and tripalmitin (3.5%, w/w) (Sigma, St. Louis, MO). Each diet was supplemented with 3.0% (w/w) fatty acid ethyl esters (Nu Chek Prep, Elysian, MN). The control diet (oleic acid diet, OA) contained 3.0% (w/w) oleic acid ethyl ester (Table 2.2). In the arachidonic acid diet (AA diet), 1.5% (w/w) of the oleic acid ethyl ester, as supplied in the OA group, was replaced by arachidonic acid ethyl ester. In the eicosapentaenoic acid diet (EPA diet), 1.5% (w/w) of the oleic acid ethyl ester as supplied in the OA group, was similarly replaced by eicosapentaenoic acid ethyl ester (EPA group). In the arachidonic acid plus eicosapentaenoic acid dietary group (AA+EPA diet), AA ethyl ester (1.5%, w/w) and EPA ethyl ester (1.5%, w/w) replaced OA ethyl ester (3.0%, w/w) (Table 2.1). Fatty acid ethyl esters were used in this study because the triglyceride and phospholipid forms of AA and EPA are unavailable. Phospholipids and triglycerides are the major sources of dietary PUFAs. During the digestion of these lipids, free PUFAs and monoglycerols are formed, and virtually all of

Table 2.1. Composition of the Diet[1]

Ingredient	Amount (g/kg diet)
Casein	200
DL-Methionine	3
Cornstarch	150
Sucrose	450
Cellulose	50
Salt Mix #200000[2]	35
Vitamin Mix #300050[3]	10
Choline Bitartrate	2
Fat[4]	100

[1] All diets contain identical amounts of carbohydrates, protein, lipids, vitamins and minerals

[2]AIN-76 salt mix contains (mg/kg diet): calcium (5200), phosphorus (4000), potassium (3600), sodium (1020), chloride (1560), sulfur (337), magnesium (507), iron (35), copper (6), manganese (54), zinc (30), chromium (2), iodine (0.2), selenium (0.1)

[3] AIN-76A vitamin mix contains (mg/kg diet): thiamine HCL (6), riboflavin (6), pyridoxine (7), niacin (30), calcium pantothenate (16), folic acid (2), biotin (0.2), cyanocobalamin (B-12, 0.1%) (10), menadione sodium bisulfite (0.8), vitamin A (4000 I.U.), vitamin E (50 I.U.), vitamin D_3 (1000 I.U.)

[4] see Table 2.2 for details.

Table 2. 2. Experimental Design[1]

	OA group[2] (n=13)	AA group[2] (n=12)	EPA group[2] (n=12)	AA+EPA group[2] (n=12)
Fat Free Diet	900	900	900	900
Safflower Oil	20	20	20	20
Olive Oil	15	15	15	15
Tripalmitin	35	35	35	35
18:1n-9 Ethyl Ester	30	15	15	0
20:4n-6 Ethyl Ester	0	15	0	15
20:5n-3 Ethyl Ester	0	0	15	15
Cholesterol	1	1	1	1
a-tocopherol (RRR)[3]	60mg	60mg	60mg	60mg
TBHQ[4]	6mg	6mg	6mg	6mg

[1] All data are expressed as g/kg diet except a-tocopherol (RRR) and TBHQ. Forty-nine animals were upon arrival randomly assigned to four dietary groups. Animals were put on chow diet for one week prior to being placed on the experimental diets for 28 days.

[2] OA group: oleic acid group; AA group: arachidonic acid group; EPA group: eicosapentaenoic acid group; AA+EPA group: arachidonic acid + eicosapentaenoic acid group.

[3] a-tocopherol (RRR) was supplemented to the diets which contained 50 I.U. vitamin E.

[4] TBHQ: tertiary butylhydroquinone

absorbed following digestion (Nelson & Ackman, 1988). Therefore, PUFAs generated during the digestion of their ethyl ester forms are assumed to be absorbed in a similar manner as PUFAs generated during the digestion of phospholipids and triglycerides. The response of dietary PUFA ethyl esters on phospholipid fatty acid composition is virtually identical to the responses observed with PUFA associated with dietary TG and phospholipids (Butcher *et al.* 1990, and Whelan *et al.* 1993).

Diets were prepared in bulk, prepackaged separately in individual Whirl-Pak bags (NASCO, Atkinsan, WI) and stored under nitrogen at -80°C. Alpha-tocopherol (RRR) (0.6 g/kg diet) (Eastman Chemical, Kingsport, TN) and tertiary butylhydroquinone (TBHQ) (60 mg/kg diet) (Eastman Chemical, Kingsport, TN) were supplemented as antioxidants to prevent oxidation of PUFA during storage. Water and food were provided *ad libitum* for 28 days. Fresh diets were provided daily and uneaten food was discarded to minimize oxidation prior to consumption. Fatty acid analysis of the diets is presented in Table 2.3.

II. Sample analysis

Stimulation of eicosanoid production with opsonized zymosan:
Opsonized zymosan was prepared by the method of Maroussen *et al.* (1985). Briefly, zymosan (10 mg) (Sigma, St. Louis, MO) was boiled in saline (0.9%, 1 mL) for 1 h, then incubated in rabbit serum (1mL, Sigma, St. Louis, MO) for

Table 2. 3. Fatty Acid Composition of the Diet[1]

	OA group	AA group	EPA group	AA+EPA group
16:0	41.4	41.0	41.0	40.0
18:1n-9	40.2	28.1	27.0	15.9
18:2n-6	18.3	18.1	18.0	17.9
20:4n-6	ND	12.8	ND	12.3
20:5n-3	ND	ND	14.0	14.1

[1] All data are expressed as g/kg diet.
ND: Not detectable

30 minutes at 37°C (Broughton *et al.* 1991). The opsonized zymosan was then washed with saline once and resuspended in saline at 2 mg/mL for storage at 4°C. Thirty minutes prior to euthanization, animals were anesthetized by methoxyflurane (Pitman-Moore, Mendelian, IL) inhalation. Following anesthetization, opsonized zymosan (1 mg, 37°C) was injected intraperitoneally to stimulate the production and release of eicosanoids from resident peritoneal macrophages. Previous studies revealed that macrophages comprise >95% of the resident peritoneal cell population in this animal model and are accessible for *in vivo* stimulation and quantitation of eicosanoid production (Whelan *et al.* 1991). Opsonized zymosan are undigestable particles which are taken up by macrophages by phagocytosis.

Analysis of leukotrienes by high performance liquid chromatography (HPLC): Thirty minutes after the zymosan injection, animals were reanethetized with methoxyflurane inhalation. Animals were killed by cervical dislocation and the peritoneal cavities were washed 3 times with a total of 10 mL cold saline containing 1 mM EDTA. The internal standard, prostaglandin B_1 (PGB_1) (100 ng), was included in the first wash. The peritoneal washes were centrifuged at 700 x g for 4 minutes. Supernatants were removed, and diluted with an equal volume of a 20% (v/v) methanol solution containing 3 mM formic acid. Ten percent of the supernatant-methanol mixture was removed for prostaglandin determination by radioimmunoassay (RIA). The leukotrienes (LTs) were separated *via* solid phase extraction using a C-18 cartridge (Burdick & Jackson, Muskegon, MI), washed once with distilled water and eluted off the cartridge with

approximately 2 mL methanol (Whelan *et al.* 1991). After being evaporated to dryness under nitrogen, the LTs were dissolved in the high performance liquid chromatography (HPLC) solvent system of methanol:water (665:335, v/v, pH 5.6) containing 5 mM ammonium acetate and 1 mM EDTA. The LTs were analyzed by reverse phase HPLC using an HP 1090 photodiode array detector system (Hewlett Packard, Atlanta, GA). The LTs were separated using a C-18 column (10 cm x 4.6 mm) (Hewlett Packard, Kennett square, PA) with a flow rate of 0.8 mL/min, monitoring at 280 nm. Peaks were identified by their distinctive UV absorption spectra, and by comparing the retention times with that of known standards. LTs were quantitated using the internal standard and known extinction coefficients of the identified compounds.

Prostaglandin and thromboxane analysis via radioimmunoassay (RIA): 6-keto PGF_{1a}, PGE_2 and TXB_2 were extracted with ethyl acetate (x3), evaporated to dryness under nitrogen and resuspended in phosphate buffered saline (0.9%) containing 0.1% gelatin. RIA was performed according to the manufacturer's instructions (Amersham, Arlington Heights, IL). Antibodies were purchased from Advanced Magnetics (Cambridge, MA) and [^{3}H] labeled antigens were purchased from New England Nuclear (Boston, MA). The standards (6-keto PGF_{1a}, PGE_2, and TXB_2) were purchased from Cayman Chemical (Ann Arbor, MI).

Analysis of fatty acid composition in liver and diet: Following the wash of the peritoneum, livers of mice were perfused with cold saline (0.9%),

removed, quick frozen in liquid nitrogen and stored at -80°C for later analysis of fatty acids. Fatty acid composition of liver phospholipids were extracted and analyzed as described previously (Whelan *et al.* 1991). Livers (100 mg) were homogenized in 0.8 mL cold saline (0.9%). Liver homogenates were resuspended in 3 mL methanol/chloroform (2:1, v/v), extracted once with chloroform/saline (1:1, v/v) and twice with chloroform. The pooled chloroform extractions were evaporated to dryness under nitrogen, and resuspended in chloroform for separation of phospholipids. Phospholipids were separated by thin layer chromatography (TLC) using HPTLC plates (10 cm x 10 cm) precoated with silica gel 60 (EM Science, Gibbstown, NJ) and chloroform/methanol (8:1, v/v) as the developing solvent. The phospholipid fractions were scraped from the TLC plates, resuspended in toluene (0.25 ml) and saponified with 0.5 N methanolic KOH at 86°C for 8 min followed by acidification with 0.7 N methanolic HCl. An internal standard, pentadecanoic acid (15:0) methyl ester (100 ng), was added to each sample prior to the saponification step. Following extraction with hexane (3x2 ml), fatty acids were methylated with ethereal diazomathane, and resuspended in hexane for gas chromatographic (GC) analysis using a Hewlett Packard 5890 series II gas chromatograph with a DB23 capillary column (0.25 mm x 30 m) (J and W Chromatography, Folsom, CA). Hydrogen was used as the carrier gas. Fatty acids were identified by comparing peaks with known standards (Nu Chek Prep, Elysian, MN), and quantified using the internal standard and the protein contents of each sample.

Protein determination: Livers were homogenized as described above. An aliquot of the liver homogenate was removed for protein analysis. Protein was determined using a modified Lowry assay (Markwell *et al.* 1985). A standard curve was generated using bovine serum albumin (Sigma, St. Louis, MO).

Statistic analysis: All data were tested for normality. ANOVA or Kruskal-Wallis ANOVA, when data were not normally distributed, was used for analysis of variance. Data with unequal variance was transformed and Fisher's Least significant difference multiple comparison method was used to determine the differences among the dietary groups. P values of linear regression was determined using the simple linear regression test. True Epistat (Epistat - Services, Richardson, Texas) and SPSS statistic package (SPSS Inc., Chicago, IL) were used to test the normality and determine the differences and linear regressions. Pooled eicosanoid data was transformed to standardize the different units of the different compounds. The transformed data are generated by subtracting the mean value of the OA control group from the experimental value, which is then divided by the standard deviation of the pooled data for each compound, *i.e.* (X-Xa)/s, where X is the experimental value, Xa is the mean value for each eicosanoid in the OA group and s is the standard deviation of the pooled (X-Xa) data for each eicosanoid. The transformed data for each eicosanoid (each animal) was pooled (*i. e.* animal #1: transformed PGE_2 + transformed TXB_2 + transformed 6-keto $PGF_{1\alpha}$ + transformed LTB_4 + transformed LTE_4). means$\pm$SEM were generated for each dietary group using the pooled

transformed data. The transformed data were tested for significance using Waller-Duncan K-ratio T test. Values were statistically different among the dietary groups at $p \leq 0.05$. The statistical results of the transformed data is presented in the Appendix (Fig A.1).

Chapter III
Results

Weight gain of the animals was not significantly different among the dietary groups. Animals in the AA and EPA groups consumed significantly more diet as compared to the other two dietary groups. However, no specific correlations could be drawn between the type of diet and the diet consumption. Weight gain and food consumption are reported in the Appendix (Table A.1).

Fatty acid composition in liver phospholipids: Tissue AA level in the AA group was significantly higher compared to the OA group (control), while tissue linoleic acid (18:2 n-6, LA) levels were significantly lower compared to the OA group (Table 3.1). N-3 PUFA content in the AA group was not significantly different compared to the OA group. Including EPA in the diet resulted in characteristic changes in tissue phospholipid fatty acid composition compared to the OA group. The hepatic phospholipids became enriched in n-3 PUFA with a concomitant decline in tissue AA levels.

When AA and EPA were simultaneously included in the diet (AA+EPA group), hepatic AA levels were more than 4 fold higher compared to the EPA group. Hepatic EPA levels declined 94% in the AA+EPA group compared to the EPA group; however, 22:6 n-3 content was 14% lower.

Table 3. 1. Fatty Acid Composition of Liver Phospholipids

	OA group	AA group	EPA group	AA+EPA group
16:0	24.29 ± 0.29[1,a] (80.33 ± 4.25)[2,a]	25.61 ± 0.34[b] (86.52 ± 5.40)[a]	26.67 ± 0.46[c] (98.31 ± 3.73)[b]	26.85 ± 0.23[c] (97.67 ± 4.52)[b]
16:1	1.05 ± 0.14[a] (3.53 ± 0.50)[a]	0.55 ± 0.17[b] (1.79 ± 0.55)[b]	0.94 ± 0.17[ab] (3.50 ± 0.68)[a]	0.34 ±0.13[bc] (1.32 ± 0.50)[b]
18:0	14.85 ± 0.22[a] (54.69 ± 3.28)[a]	16.86 ± 0.49[b] (63.50 ± 5.05)[ab]	15.05 ± 0.15[a] (62.16 ± 2.80)[ab]	16.53 ± 0.19[b] (67.09 ± 3.48)[b]
18:1n-9	13.66 ± 0.25[a] (49.93 ± 2.94)[a]	8.76 ± 0.39[b] (32.54 ± 2.31)[bc]	10.06 ± 0.21[c] (38.50 ± 3.16)[c]	6.66 ± 0.16[d] (26.62 ± 1.23)[b]
18:2n-6	15.13 ± 0.38[a] (55.15 ± 3.63)[a]	12.68 ± 0.48[b] (47.38 ± 3.78)[a]	19.15 ± 0.62[c] (79.48 ± 3.69)[b]	14.08 ± 0.25[a] (56.42 ± 3.33)[a]
20:3n-6	1.86 ± 0.12[a] (7.34 ± 0.52[a])	ND ND	0.98 ± 0.20[b] (4.38 ± 0.94)[b]	ND ND
20:4n-6	21.29 ± 0.32[a] (84.24 ± 5.32)[a]	26.33 ± 0.43[b] (106.03 ± 7.49)[b]	5.19 ± 0.19[c] (22.80 ± 1.37)[c]	22.01 ± 0.23[a] (95.23 ± 4.69)[ab]
20:5n-3	ND ND	ND ND	6.53 ± 0.27[a] (28.67 ± 2.02)[a]	0.41 ± 0.13[b] (1.80 ± 0.57)[b]
22:4n-6	ND ND	0.55 ± 0.14 (2.53 ± 0.71)	ND ND	ND ND
22:5n-6	1.03 ± 0.19[a] (4.48 ± 0.82)[a]	1.96 ± 0.28[b] (8.57 ± 1.31)[b]	ND ND	ND ND
22:5n-3	ND ND	ND ND	2.37 ± 0.29[a] (11.49 ± 1.53)[a]	2.38 ± 0.13[a] (11.36 ± 0.99)[b]
22:6n-3	6.84 ± 0.24[a] (29.19 ± 1.99)[a]	6.70 ± 0.15[a] (28.99 ± 1.94)[a]	12.49 ± 0.34[b] (59.00 ± 2.53)[b]	10.72 ± 0.27[c] (50.15 ± 2.81)[c]
Total (n-3) FA	6.84 ± 0.24[a] (0.29 ± 0.02)[a]	6.70 ± 0.15[a] (0.29 ± 0.02)[a]	21.39 ± 0.47[b] (0.99 ± 0.05)[b]	13.52 ± 0.20[c] (0.63 ± 0.04)[c]
Total (n-6) FA	39.32 ± 0.33[a] (1.51 ± 0.09)[a]	41.51 ± 0.29[b] (1.64 ± 0.12)[a]	25.82 ± 0.59[c] (1.07 ± 0.05)[b]	36.09 ± 0.26[a] (1.52 ± 0.08)[a]
(n-3)/(n-6) ratio	0.17 ± 0.01[a] (0.19 ± 0.01)[a]	0.16 ± 0.01[a] (0.18 ± 0.01)[a]	0.84 ± 0.04[b] (0.94 ± 0.04)[b]	0.38 ± 0.01[c] (0.42 ± 0.01)[c]

[1] Data are mole% and are expressed as mean±SEM. Values with different letters in the same row are significantly different at $p<0.05$.

[2] Data in parentheses are mg/100mg protein and are expressed as mean±SEM.

ND: not detectable

Eicosanoid production from stimulated peritoneal macrophages in vivo: LTB_4 production *in vivo* was 2 fold higher in the AA group compared to the OA group (control) (Table 3.2). While EPA fed animals produced no detectable levels of LTB_4, including AA in the diet containing EPA (AA+EPA group) resulted in LTB_4 levels which were comparable to that in the AA group. LTE_4 production in the AA group was not significantly different from the OA group. LTE_4 production in the EPA group was significantly lower compared to all other dietary groups. However, the combination of AA+EPA feeding significantly increased LTE_4 production to a level intermediate to that of the AA ($p<0.08$) and EPA ($p<0.05$) groups, respectively. LTE_5 was only detected in the dietary groups which included EPA. LTE_5 production was 82% lower in the AA+EPA group compared to the EPA group (Table 3.2).

Similarly, PGE_2 and TXB_2 levels were highest in the AA group compared to the OA group; however, these differences were not statistically significant ($p<0.08$ and $p<0.06$, respectively) (Table 3.2). PGE_2 and TXB_2 production were significantly higher in the AA and AA+EPA groups compared to the EPA group (Table 3.2). 6-keto $PGF_{1\alpha}$ production (the stable product of PGI_2) was significantly higher in the AA group and lower in the EPA group compared to the OA group (Table 3.2). Including AA to the diet containing EPA (AA+EPA group) resulted in a 6-keto $PGF_{1\alpha}$ level which was equivalent to that of the AA group.

When total eicosanoid production was pooled and standardized compared to the OA group, the pooled eicosanoid formation was significantly

Table 3. 2. Effect of Dietary Unsaturated Fatty Acids on Eicosanoid Production in Peritoneal Macrophages *In Vivo* Stimulated with Opsonized zymosan[1]

	OA group (n=8)	AA group (n=8)	EPA group (n=10)	AA+EPA group (n=9)
LTB_4	3.56 ± 1.14^{a}	6.64 ± 0.73^{b}	ND	6.14 ± 0.65^{b}
LTE_4[2]	83.65 ± 11.24^{a}	101.39 ± 11.30^{a}	38.31 ± 5.63^{b}	75.56 ± 7.64^{a}
6-Keto PGF_{1a}	4.79 ± 0.85^{a}	8.27 ± 0.88^{b}	0.63 ± 0.11^{c}	9.09 ± 1.03^{b}
PGE_2	0.73 ± 0.09^{ab}	1.03 ± 0.12^{a}	0.55 ± 0.07^{b}	0.90 ± 0.14^{a}
TXB_2	0.18 ± 0.02^{ab}	0.31 ± 0.07^{a}	0.15 ± 0.04^{b}	0.27 ± 0.04^{a}
LTE_5	ND	ND	47.44 ± 9.64^{a}	8.39 ± 1.85^{b}

[1] All data are ng/mouse and are expressed as mean±SEM. Values with different letters on the same row are significantly different at $p<0.05$. Resident peritoneal cells were stimulated by intraperitoneal injection of opsonized zymosan. The peritoneal washes were collected and analyzed for LT and PG. LT were analyzed by HPLC and PG were analyzed using RIA.

[2] Data includes 11-trans LTE_4

higher in the AA and AA+EPA dietary groups compared to the OA and EPA groups, and lowest in the EPA group compared to all other dietary groups (Fig 3.1).

Correlation of eicosanoid production and phospholipid AA content: All eicosanoids produced *in vivo* were significantly correlated with hepatic phospholipid AA content (Table 3.3). LTB_4, LTE_4, 6-keto $PGF_{1\alpha}$, PGE_2 and TXB_2 were positively correlated with AA, while LTE_5 was negatively correlated with hepatic phospholipid AA content. It should be noted that while fatty acid composition data for the resident peritoneal cells was unable to be obtained, previous studies have demonstrated that composition changes in hepatic phospholipids strongly reflect fatty acid changes in other tissues, including resident peritoneal macrophages (Whelan *et al.* 1991, 1992 & 1993).

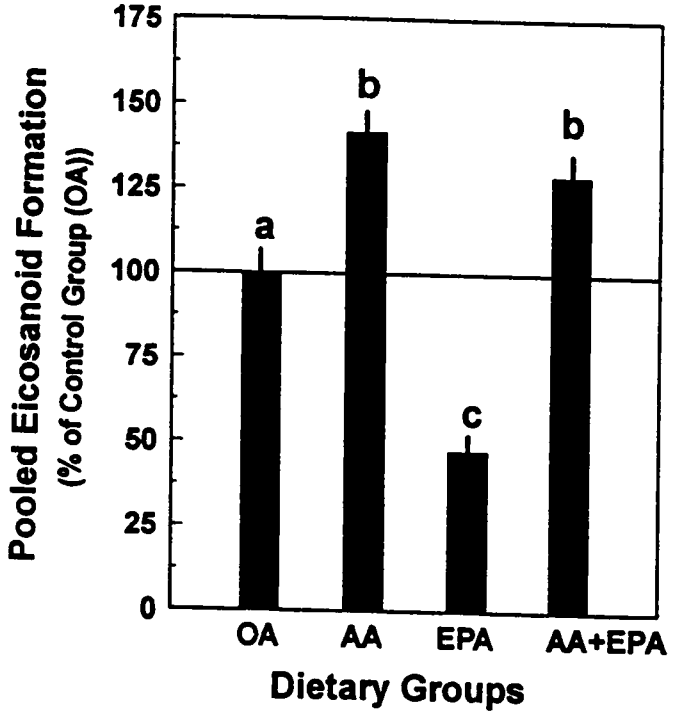

Fig. 3.1. Effect of different types of unsaturated fatty acids on pooled eicosanoid formation. Dietary AA significantly elevates tissue eicosanoid formation ($p<0.05$), while dietary EPA suppresses eicosanoid formation ($p<0.05$). Including AA in the same diet with EPA, replenishes eicosanoids which were similar to that observed in the dietary group containing AA-only. The transformed data were tested for significance using Waller-Duncan K-ratio T test. Values were statistically different among the dietary groups at $p\leq0.05$ (see methods for a complete description)

Table 3.3. Relationship of Eicosanoid Production and Tissue AA Content[1]

	Correlation Coefficient (r)	level of significance (p)
LTB_4	0.22	<0.001
LTE_4	0.74	<0.001
6-keto-PGF_{1a}	0.76	<0.001
PGE_2	0.50	<0.003
TXB_2	0.41	<0.015
LTE_5	-0.68	<0.001

[1] Eicosanoid levels of each animal were correlated with the AA content in liver phospholipids. Significance level of the correlation was determined using a simple linear regression test.

Chapter IV
Discussion

AA is an important constituent of membrane phospholipids. When it is released it can be oxidatively metabolized to eicosanoids, a group of hormone-like, signaling agents which affect cell behavior and cell communication. When eicosanoids are produced chronically and excessively, they can be proinflammatory, proatherogenic and prothrombotic (Kinsella and Lokesh 1990, Kinsella *et al.* 1990a & 1990c, Leaf and Weber 1988). In order to reduce the potential risks of excess eicosanoid formation, it is necessary to regulate the amount of eicosanoids produced. It has been repetitively demonstrated that altering tissue phospholipid AA content is an important means of regulating eicosanoid formation (Whelan *et al.* 1991 &1993, Broughton *et al.* 1991, and Mann *et al.* 1993). Since altering the dietary levels of LA, AA, and EPA can alter tissue phospholipid AA content, changes in the dietary levels of these PUFAs can be used to regulate eicosanoid formation.

I. Effects of dietary unsaturated fatty acids on their incorporation into membrane phospholipids

Dietary LA, the metabolic precursor of AA, is an important contributor to tissue AA content. It is converted *de novo* to AA through elongation and desaturation via Δ6, and Δ5 desaturases with the Δ6 desaturase being the rate limiting enzyme (Fig 1.3) (Brenner 1977). The conversion of LA to AA is

highly regulated through product feedback inhibition (Brenner *et al.* 1969, and Brenner & Peluffo 1969). When consumed at low levels (< 2 en%), LA effectively contributes to tissue AA content, but at higher intakes, dietary LA appears to have little effect (Mohrhauer and Holman 1963b & 1963c, Whelan *et al.* 1992, James 1993) . Additionally, the rate of conversion of LA to AA is even lower in humans than in rodent models (James 1993, De Gómez Dumm & Brenner 1977). Therefore, dietary LA at levels greater than 2 en% may not play an important role in modulating tissue AA content (Lands, Emken 1988, Whelan 1992, James 1993).

Recently, it has been demonstrated that dietary AA can rapidly and effectively enrich tissue phospholipids with AA (Fig 1.4). Hepatic phospholipid AA content was shown to be elevated when hamsters were put on a diet containing AA for 4 weeks as compared to hamsters that were on OA or LA containing diets (Whelan *et al.* 1993). Similar results were observed in this current study. AA feeding (1.5%, w/w) resulted in significantly higher levels of AA in membrane phospholipids as compared to the animals fed the OA control diet (Table 3.1). AA has been shown to be enriched in membrane phospholipids of liver, lung, heart, spleen, kidney, testes and peritoneal macrophages when diets of animals are supplemented with AA (Whelan *et al.* 1993, Iritani and Narita 1984, and Danon *et al.* 1975). In rats, hepatic phospholipid AA content was shown to respond to dietary AA levels in a dose dependent manner (Mohrhauer and Holman 1963a & 1963b). Moreover, it was also demonstrated in human subjects that intake of AA elevated the circulating level of AA (Seyberth *et al.* 1975, Sinclair *et al.*

1987, O'Dea and Sinclair 1982, Butcher *et al.* 1990). These results support the hypothesis that dietary AA may play a more important role in regulating tissue AA levels and eicosanoid formation versus dietary LA, even though the consumption of LA is 100 times greater than that of AA (*i.e.* 20g/d vs. 0.16-0.22g/d) (Nottage 1992, and Whelan *et al.* 1993), . However, it should be noted that this conclusion has yet to be proven kinetically and is limited to the highly defined circumstances in which these studies have been performed (Danon *et al.* 1975, Whelan *et al.* 1992, Whelan *et al.* 1993, Mann *et al.* 1994).

Dietary n-3 PUFA can also effectively alter tissue AA content. n-3 PUFA can effectively enrich tissue phospholipids with n-3 PUFA, primarily at the expense of tissue AA (Fig 1. 5). EPA is a 20 carbon fatty acid analogue of AA in the n-3 PUFA family. Due to its structural similarity with AA, EPA works as an effective competitor of AA. Dietary EPA competes with AA for incorporation into tissue phospholipids and formation of eicosanoids. Dietary EPA remarkably suppresses tissue phospholipid AA content and AA-derived eicosanoid formation when it is provided in the absence of dietary AA. Concomitantly, tissue phospholipid n-3 PUFA and EPA content (Whelan *et al.* 1993, Broughton *et al.* 1991) increases with a subsequent increase in EPA-derived eicosanoids (Whelan *et al.* 1991 & 1993). Therefore, it appears that the effects of AA and EPA are diametrically opposed. However, prior to this study, the relationship between dietary AA and EPA had yet to be directly evaluated. The current study is the first to systematically investigate

the antithetic effects of dietary AA and EPA on fatty acid composition and eicosanoid metabolism.

When AA and EPA were included in the same diet (AA+EPA diet), tissue phospholipid AA content was replenished when compared to the EPA feeding group which suppressed phospholipid AA content four fold. Tissue phospholipid EPA was ten times lower in mice on an AA+EPA diet when compared to mice on an EPA-only diet (Table 3.1). Similar results were observed in an earlier study by O'Dea and Sinclair using a diet of tropical fish (O'Dea and Sinclair 1982). After the subjects were fed the tropical fish diet, their plasma AA levels increased. It had been expected that the plasma AA levels would decrease because the fish were rich in n-3 PUFA. However, the opposite result occurred, because the fish were also rich in AA. The same group repeated their experiment involving tropical fish with a low fat diet (7 en%) and reported the same effect as observed in their previous study (Sinclair *et al.* 1987, and Butcher *et al.* 1990). In the current study, equal amounts of AA and EPA, which together provided approximately 6% of calories, were provided in the diet. It was observed that AA was more favorably incorporated into membrane phospholipids than EPA. A similar result was also reported recently by Nilsson *et al.* (1992) in a study involving two different radioisotopes. In this study, animals were given [^{14}C]-labeled AA and [^{3}H]-labeled EPA intragastrically. Recovery of [^{14}C]-label in tissue phospholipids was higher than the recovery of [^{3}H]-label, suggesting that more AA or its metabolites were incorporated into tissue phospholipids

compared to EPA or its metabolites. Of importance, these differences were observed within 1 hr after the administration of the radiolabeled compounds.

Data from this present study clearly demonstrates that the competition between AA and EPA favors AA. There are several mechanisms which might explain this phenomenon. First, AA appears to be preferentially incorporated into tissue phospholipids compared to EPA (Nilsson *et al.* 1992). In the current study, the distribution of AA in phospholipids exceeded that of EPA when they were both provided in the same diet. AA and EPA have been suggested to share the same esterification system in skin fibroblast and intact mouse peritoneal macrophages (Banerjee and Rosenthal 1985, Fernánedez *et al.* 1992). This esterification system appears to have a higher selectivity for AA than for EPA (Iritani *et al.* 1984). Also, when rats adapted to a corn oil or a fish oil diet were switched to a fat free diet, their tissue AA content appeared to decrease slower than their tissue EPA content (Iritani and Narita 1984, and Iritani *et al.* 1987). Therefore, it has been suggested that both incorporation of the fatty acids into the membrane phospholipids, and their release from the membrane phospholipids favors AA compared to EPA. Second, AA is a poor substrate for elongation and desaturation compared to EPA. The chain elongation of EPA (20:5n-3) to DHA (22:6 n-3) is distinctly more pronounced than that of AA to its elongation metabolites, while the equilibrium between AA and its elongation products is shifted in favor of AA (Rosenthal *et al.* 1991, Hagve and Christophersen 1984). At the same time, retroconversion of the respective C_{22} fatty acid (22:4 n-6) to AA (20:4 n-6) is significantly higher (41%) than that of DHA (22:6 n-3) to EPA (20:5 n-3) in

cultured human skin fibroblast (Rosenthal *et al.* 1991). Thus, AA (20:4 n-6) and DHA (22:6 n-3) tend to become accumulated in tissue phospholipids. Third, AA is a poor substrate for ß-oxidation. Both AA and EPA are poorly oxidized when compared to shorter chain fatty acids which are better sources for energy (Hiltunen *et al.* 1986). It appears that the very long chain PUFA (C_{20}) are more poorly oxidized than their precursors (C_{18}). For example, the oxidation rate of AA is about 30% less than that of LA (Leyton *et al.* 1987).

II. Effects of dietary unsaturated fatty acids on eicosanoid production

Tissue AA is an important component in membrane phospholipids and is the major precursor of eicosanoids. Increasing tissue AA content significantly elevates eicosanoid formation in mice, hamster and rat models (Whelan *et al.* 1991 & 1993, Mann *et al.* 1994). Culturing human endothelial cells in AA-rich media was also shown to enhance the production of eicosanoids *i.e.* prostacyclin (Spector *et al.* 1983).

When AA is included in the diet, production of AA-derived eicosanoids increased following *in vivo* stimulation with opsonized zymosan (Table 3.2). Animals in the AA dietary group produced higher levels of LTB_4 and PGI_2 (measured as 6-keto $PGF_{1\alpha}$) compared to the OA control group. Similarly, the increases in PGE_2 and TXB_2 production in the AA group also approached significance ($p<0.08$ and $p<0.06$, respectively) when compared to the OA group. These results are consistent with those published previously (Seyberth *et al.* 1975). In the earlier study by Seyberth *et al.*, four

human subjects consuming a diet of 6 grams of AA ethyl ester were reported to have elevated levels of a urinary PGE_2-metabolite in urine. Similarly, PGI_2 and TXA_2 production were shown to be elevated in the aorta of rats fed ethyl arachidonate (90 mg/day) for two weeks (Steel *et al.* 1990). PGE_2 and the lipoxygenase product 12-HETES formation, following *in vitro* stimulation of macrophages and platelets, were shown to be elevated as a result of feeding an AA diet to Syrian hamsters (Whelan *et al.* 1993). Recent data generated from humans also demonstrated that subjects consuming AA-containing fish (500 g/d) had increased platelet function (Butcher *et al.* 1990). Therefore, dietary AA administration can not only result in significant increases of tissue eicosanoid production but also alter the physiological effects that are associated with eicosanoids.

Dietary EPA, on the other hand, serves as very good antagonist to AA-derived eicosanoids, and is a source of EPA-derived eicosanoids. In the current study, AA-derived eicosanoids were significantly lower in the EPA group as compared to the AA group with a concomitant increase in the concentration of EPA-derived LTE_5 (Table 3. 2). This effect of dietary EPA on eicosanoid formation has been reported previously (Whelan *et al.* 1993). Increasing dietary n-3 PUFA level or (n-3)/(n-6) ratio was shown to decrease the production of AA-derived eicosanoids and increase EPA-derived eicosanoid content *in vivo* and *in vitro* (Broughton *et al.* 1991, Hwang *et al.* 1988, and Whelan *et al.* 1991&1993). Thus, EPA is very potent in diminishing the formation of AA-derived eicosanoids in the absence of dietary AA.

Therefore, because the actions of dietary AA and EPA are diametrically opposed, the current study was designed to evaluate, in part, the antithetic relationship of dietary AA and EPA on eicosanoid production, and whether dietary AA could abrogate the effect of dietary EPA.

In this study, the opposing effects of dietary AA and EPA were systematically evaluated using diets containing equivalent amounts of AA and EPA. When AA was added to an EPA containing diet (AA+EPA), the production of AA-derived eicosanoids was similar to that of the AA-only group, and was significantly higher than the EPA-only group. While dietary EPA alone is effective in decreasing the AA-derived eicosanoids, the presence of dietary AA eliminated the effects of EPA. Even though EPA is as good a substrate as AA for the 5-LOX (Ochi *et al.* 1983) and a very good inhibitor for PGHS (Culp *et al.* 1979), it is unable to effectively compete with dietary AA for the formation of eicosanoids. For example, TXB_2 production was 50% lower in the EPA group compared to the AA group. However, TXB_2 production was replenished when AA was added back to the diet that contained EPA. Similarly, LTB_4 formation was nondetectable in the EPA group, while adding AA back to the diet containing EPA increased its formation to the same level as what was observed in the AA group. AA+EPA feeding resulted in higher AA-derived eicosanoid formation and lower EPA-derived eicosanoid formation as compared to the EPA group. This data suggests that dietary AA is a more potent antagonist towards EPA than EPA is towards AA. The pooled eicosanoid data (Fig 3. 1) effectively summarizes the antithetic effect of dietary AA and EPA. This figure emphasizes the ability

of dietary AA to significantly increase AA-derived eicosanoids even in the presence of an equivalent amount of dietary EPA.

The underlying mechanisms responsible for the effects of AA on eicosanoid production have yet to be ascertained; however, data from this study suggests that it is in part related to tissue AA levels. The significant correlation of hepatic AA levels with the production of each AA-derived eicosanoid suggest that AA level is an important factor in regulating eicosanoid formation (Table 3. 3). However, while it is understood that a cause and effect relationship can not be established with correlation coefficients, similar results have been reported by Steel *et al.* (1993). This group reported parallel increases in tissue AA content and cell-stimulated eicosanoid production with dose dependent increases of dietary AA. Therefore, it appears that altering dietary AA levels modulate tissue AA content, and is an important regulator of eicosanoid formation.

In conclusion, based on the evidence presented in this paper and the evidence of others (Whelan *et al.* 1993, Nilsson *et al.* 1992, Sinclair *et al.* 1987, Butcher *et al.* 1990, Steel *et al.* 1991 & 1993, Mann *et al.* 1994), we propose the following metabolic scheme (Fig 4.1). Dietary AA+EPA feeding suppresses the effects of EPA on lipid composition and eicosanoid formation and enhances the effects of AA. When AA is included in the diet alone, it enriches tissue AA content and elevates eicosanoid formation. When EPA is included in the diet alone, it decreases tissue AA content and suppresses

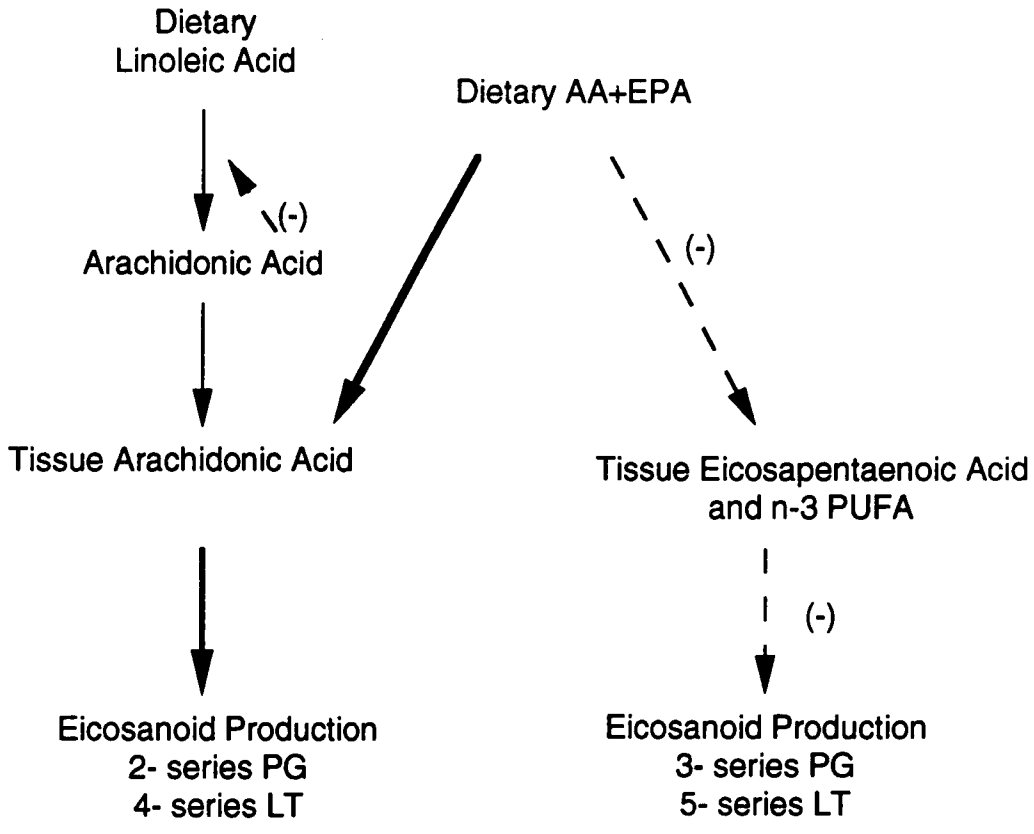

Fig 4.1 Dietary arachidonic acid (AA) abrogates the effects of dietary eicosapentaenoic acid (EPA). When AA and EPA are included in the diet simutanously, AA diminishes the inhibition effects of EPA upon the incorporation of AA into phospholipids and the formation of AA-derived eicosanoid formation. Feeding of AA+EPA has a similar effect as AA feeding only. Tissue AA content and AA-derived eicosanoid formation were augmented as if EPA was not included in the diet. PUFA, polyunsaturated fatty acids; PG, prostanoids; LT, leukotrienes. Solid lines represent normal metabolic pathway; dotted lines show inhibition or negative effect; and bold solid lines show enhancement of pathways or a positive effect.

AA-derived eicosanoid formation (Whelan *et al.* 1993). When equivalent levels of AA and EPA are included in the diet, AA has the greater of the two effects. That is, tissue AA content will increase, thereby increasing AA-derived eicosanoid production, and diminish the ability of dietary EPA to alter tissue fatty acid composition and eicosanoid production. Therefore, the ability of dietary AA to abrogate the effects of dietary EPA suggests that dietary AA may also compromise the physiological effects associated with the consumption of n-3 PUFA.

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Literature Cited

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Appendix

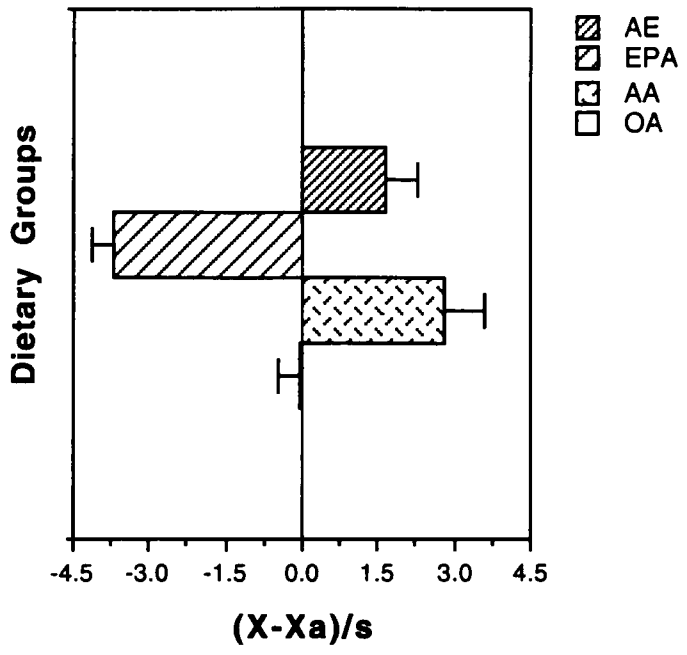

Fig A.1. Statistical analysis of the pooled eicosanoid production after transformation of the original data. Eicosanoid data were pooled and transformed to eliminate the different units of the various compounds. The data was transformed (X-Xa/s) as described previously (see Materials and Methods section for complete description). The transformed data were tested for significance using Waller-Duncan K-ratio T test, where $p \leq 0.05$ was considered to be statistically significant. Data were presented as mean$\pm$SEM. Bars with different letters are statistically different from each other at $p \leq 0.05$. X is the individual eicosanoid, while Xa is the mean of the OA control group, s is the standard deviation (see Materials and Methods section for detail description).

Table A.1 Weight gain and food consumption

	OA Group (n=13)	AA Group (n=12)	EPA Group (n=12)	AA+EPA Group (n=12)
Weight Gain[1]	21.5 ± 2.9	22.3 ± 7.1	22.6 ± 3.1	20.1 ± 5.7
Consumption of food[2]	13.2 ± 2.1[a]	14.4 ± 2.1[b]	14.4 ± 2.6[b]	13.1 ± 1.6[a]

[1] Weight gain of animals were reported as g/cage, three mice were caged together in each cage.

[2] Food consumption of animals were reported as g/cage/day. Food consumption were monitored and recorded daily.

[a,b] Values in the same row with different superscripts are significantly different from each other at $p \leq 0.05$.

Vita

Bangyan Li (married name, Bangyan Li Stiles) was born in Shenyan, Liaoning province, People's Republic of China on June 21, 1968. She attended elementary school in the Railroad Primary School, and graduated from there in 1980. She graduated from the Pei Zheng Middle school in Guangzhou, and attended the high school affiliated with the South China Normal University. After graduating from high school, she attended the Jinan University (Guangzhou) in 1986, and received her Bachelor's of Science degree in Food Chemistry. In 1991, she came to the United State to pursue her Master's Degree at The University of Tennessee, Knoxville. In August 1994, she received her Master of Science degree in Nutritional Science.

She is currently a Ph. D. student of the Nutrition Department in The University of Texas at Austin, and works as a research assistant in the M. D. Anderson Cancer Center affiliated with The University of Texas.

List of Publications:

1. Stiles, B. L. , Birdwell, C. B., and Whelan, J. (1994) The antithetic relationship of dietary arachidonic acid and eicosapentaenoic acid. J. Lipid Res. (in press-Sept. 1994).

2. Whelan, J., Stiles, B. L., Surette, M., and Bailey, J. W. (1994) Evidences that dietary AA may increase circulating triglycerides. Biochem. Biophys. Res. Comm. (in review)

3. Stiles, B. L. , Cha, Y.-S., Sachan, D. S., and Whelan, J. (1994) Dietary docosahexaenoic acid decreases plasma triglycerides with mixed effects on indices of ß-oxidation. J. Nutritional Biochemistry (in review).

4. Whelan, J., Li, B. Y., and Birdwell, C. (1994) Dietary Arachidonic acid increases eicosanoid production even in the presence of equal amounts of eicosapentaenoic acid, in: the Proceedings of the Third International Conference on Eicosanoids and other Bioactive Lipids in Cancer, Inflammation and Radiation Injury, (K. W. Honn, L. J. Marnett, and S. Nigam, eds.), Peragom Press.

5. Liu, H-K., Chen, Z-L., Li, B. Y., and Xia, W. (1991) Determination of Arginine by Differential Pulse Voltammetry. Journal of Jinan University - Natural Science & Medicine Edition. 12(additional number): 12-15.

6. Li, B.Y. Functions and Metabolisms of Micro Element Zinc: A Review. Journal of Jinan University. Natural Science & Medicine Edition. 11(3): 89-92.

List of Abstracts:

1. Li, B. and Whelan, J. (1994) The antithetic relationship of dietary arachidonic acid and eicosapentaenoic acid on eicosanoid production *in vivo*. FASEB J. 8 (5) Part II: 5355.

2. Whelan, J., Li, B. Y., and Surette, M. E. (1994) Evidence that dietary arachidonic acid elevates circulating triglyceride levels. FASEB J. 8 (5) Part II: 4072.

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4. Whelan, J., Li, B. Y., and Surette, M. E. (1993) Evidence that dietary arachidonic acid elevates plasma triglycerides. Conference on Nutrition and Biotechnology in Heart Disease and Cancer. Chapel Hill, NC, December, 1993.

5. Li, B, Cha, Y.-S., Sachan, D. S., and Whelan, J. (1993) Dietary docosahexaenoic acid decreases plasma triglycerides with mixed effects on indices of ß-oxidation. FASEB J. 7 (5): 999.