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Increasing Dietary Linoleic Acid Does Not Increase Tissue Arachidonic Acid Content in Adults Consuming Western- Type Diets

Brian Rett
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To the Graduate Council:

I am submitting herewith a thesis written by Brian Rett entitled "Increasing Dietary Linoleic Acid Does Not Increase Tissue Arachidonic Acid Content in Adults Consuming Western- Type Diets." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Jay Whelan, Major Professor

We have read this thesis and recommend its acceptance:

Hollie Raynor, Michael McEntee

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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A Thesis
Presented for the
Master of Science Degree
The University of Tennessee, Knoxville

Brian Rett
May 2011

DEDICATION

I would like to dedicate this thesis to my family for all of their support

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I would like to thank Dr. Jay Whelan for his support and guidance throughout this project.

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ABSTRACT

Linoleic acid, with a DRI of 12-17g/d, is the most highly consumed polyunsaturated fatty acid in the Western diet and is found in virtually all commonly consumed foods. The concern with dietary linoleic acid, being the metabolic precursor of arachidonic acid, is its consumption may enrich tissues with arachidonic acid and contribute to chronic and overproduction of bioactive eicosanoids. However, no systematic review of human trials regarding linoleic acid consumption and subsequent changes in tissue levels of arachidonic acid has been undertaken. In this study, we reviewed the human literature that reported changes in dietary linoleic acid and its subsequent impact on changing tissue arachidonic acid in erythrocytes and plasma/serum phospholipids. We identified, reviewed, and evaluated all peer-reviewed published literature presenting data outlining changes in dietary linoleic acid in adult human clinical trials that reported changes in phospholipid fatty acid composition (specifically arachidonic acid) in plasma/serum and erythrocytes within the parameters of our inclusion/exclusion criteria. Decreasing dietary linoleic acid up to 90% was not significantly correlated with changes in tissue arachidonic acid levels ($p=0.39$). Similarly, when dietary linoleic acid levels were increased six fold, no significant correlations with tissue arachidonic acid levels were observed ($p=0.72$). However, there was a positive relationship between dietary gamma-linolenic acid and arachidonic acid on changes in tissue arachidonic levels. Our results do not support the concept that modifying current intakes of dietary linoleic acid has an effect on changing tissue levels of arachidonic acid in adults consuming Western-type diets.

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ABBREVIATIONS

AA	**Arachidonic acid**
AI	**Adequate intake**
CVD	**Cardiovascular disease**
DRI	**Dietary reference intake**
EFA	**Essential fatty acid**
ER	**Endoplasmic reticulum**
GLA	**Gamma linolenic acid**
LA	**Linoleic acid**
TAG	**Triacylglycerol**
PL	**Phospholipid**

Chapter 1

Introduction

1.1 Dietary Lipids: Digestion, Absorption and Transportation

Triacylglycerols (TAG), followed by phospholipids (PL), are the major source of dietary lipids in the human diet. Digestion of lipids begins in the mouth with partial hydrolysis of *sn-3* fatty acids of TAG by lingual lipase (1), and subsequently by gastric lipase in the stomach. Along with gastric lipase, the stomach aids digestion with gastric grinding and the result is a mixture of emulsified components containing lipids and the enzymatic products (2). The emulsion enters the small intestine where digestion of lipids continues. Pancreatic lipase (in the presence of colipase) cleaves fatty acids from the *sn-1* and *sn-3* positions of TAG (2). Phospholipids are acted upon by pancreatic phospholipase A_2 at the *sn-2* position generating free fatty acids (typically a polyunsaturated fatty acid) and lysophospholipids. Cholesterol esters are acted upon by cholesteryl esterase forming free fatty acids and unesterified cholesterol.

In order for these hydrophobic products of digestion to be absorbed by intestinal mucosal cells, they have to become sufficiently soluble within the aqueous environment to cross the unstirred water layer (3). For this to happen, aggregates of fatty acids, cholesterol, lysophospholipids and monoacylglycerides are combined with bile salts and acids to form micelles (10-12).

These small emulsified aggregates of lipid products transverse the unstirred water layer and are absorbed by enterocytes passively or by active transport. The active transporter for fatty acids, FATP4, is responsible for absorption when concentrations of fatty acids are low (4), but at high concentrations, diffusion is the predominant mechanism. After absorption, fatty acids are delivered to the endoplasmic reticulum (ER) of the enterocyte via fatty acid binding proteins (5) where they are re-esterified into TAG, cholesterol esters and phospholipids. Along with

cholesterol and apoproteins, these lipid components are incorporated into chylomicrons for transport in the general circulation (6).

The fatty acids from the TAG of the chylomicrons are delivered to extrahepatic tissues via the action of lipoprotein lipase (through the generation of two free fatty acids and a monoacylglycerol). Loss of TAGs results in remodeling of the chylomicron where surface components containing phospholipids, cholesterol and cholesterol esters form the backbone of other lipoproteins (i.e., high density lipoproteins), resulting in a chylomicron remnant that is subsequently cleared by the liver. The liver either uses (metabolizes) these lipid components or repackages them with endogenously synthesized lipids from the endoplasmic reticulum into very low density lipoproteins (VLDL) for delivery to extrahepatic tissue. While in the plasma, the TAGs from the VLDL can follow a similar metabolic path as that of the chylomicron. The resulting remnant can pick up cholesteryl esters from high density lipoproteins in its conversion to low density lipoproteins or be cleared by receptors in the liver. The re-circulated lipids associated with lipoproteins are the source of fatty acids for plasma phospholipids and represent those lipids derived from the endoplasmic reticulum of hepatocytes (7, 8).

1.2 Essential Fatty Acids.

Evidence for the essentiality of certain types of fats emerged in the early 1900's when a lipid-deficiency disease was reported in rodents. Rodents fed lipid-free diets developed clinical symptoms such as hindered growth, weight loss and dermatitis (9). Dermatitis is a skin condition that typically exhibits red, scaly skin and dandruff, and can manifest itself with dermal lesions (9). Feeding a lipid source, i.e. lard, rescinded these symptoms and restored growth. From this experiment, it appeared that dietary fat, like vitamins, played a unique role in supporting basic physiological systems. However, lard

contains a variety of saturated, monounsaturated and polyunsaturated fatty acids and necessitated research with other oils and individual fatty acids to discern the curative effects. In subsequent studies, corn oil and linseed oil also alleviated the symptoms, while butter and coconut oil failed to do so (10). Further work found that feeding linoleic acid (LA) and arachidonic acid (AA), in isolation, cured these clinical symptoms, although a lower amount of AA was needed (11).

The rodent observations of essential fatty acids resembled human observations. Confirmation of deficiency symptoms were seen in adult males given LA-deficient intravenous therapy, and LA-containing oils alleviated these symptoms (12). Similarly, evidence emerged when infants receiving intravenous therapy developed similar EFA-deficiency symptoms and they also were cured with methyl-linoleate (13). These combined results suggested a common requirement of LA for rodents and humans.

1.3 Polyunsaturated Fatty Acids

Essential fatty acids belong to a larger family, called polyunsaturated fatty acids (PUFA). PUFA are a group of fatty acids that have more than one double bond (14). They are divided into several families, depending upon the location of the terminal double bond closest to the methyl end, i.e., double bonds located 3, 6, or 9 carbons from the terminal methyl carbon These families are non-interconvertible, since mammals lack the ability to introduce double bonds beyond the ninth carbon from the carboxylic acid end (15). One of the families is the n-6 fatty acids. The designation for this family is due to the terminal double bond at the sixth carbon from the methyl end. Of all the dietary PUFAs, the n-6 family is consumed in the greatest amount in the Western diet (16).

1.4 Dietary n-6 PUFA and Tissue Arachidonic Acid

The parent compound of n-6 PUFA family is LA (18:2n-6). LA is ubiquitous in nearly all commonly consumed foods in the Western diet and is also the predominant PUFA in foods (60%-100% of total PUFA content in virtually all commonly consumed foods). LA is found in the highest concentrations in vegetable oils, nuts and legumes, although significant amounts are also found meat, eggs and animal products. Once consumed, LA and other n-6 PUFAs can be delivered to tissues, as described previously, and in incorporated into the *sn-2* position of phospholipids by a variety of acyltransferases (17). Following consumption, LA can also be converted to downstream n-6 fatty acids following the action of delta-6 desaturase (Figure 1). This enzyme is the rate-limiting step in the formation of more highly unsaturated fatty acids. LA is converted to gamma-linolenic acid (GLA, 18:3 n-6) via the delta-6 desaturase, which is immediately elongated to di-homo-gamma-linolenic acid (DGLA, 20:3 n-6) with the addition of two carbons by an elongase. AA (20:4n-6) is formed from DGLA via the action of delta-5 desaturase, with the addition of a double-bond between the 5^{th} and 6^{th} carbons.

Of these n-6 PUFA, LA and AA are preferentially incorporated into membrane phospholipids. Phospholipids are polar lipids that form the non-hydrophilic barrier of the cell membrane. Their unique properties are a result of a hydrophilic head, containing a phosphoester bond linked to a charge (choline, serine, inositol, ethanolamine) and two hydrophobic fatty acid tails, attached by ester bonds. The *sn-1* position is reserved for saturated and monounsaturated fatty acids, and the *sn-2* position is reserved for PUFA(18). The remodeling of fatty acid composition of phospholipids is carried out by a variety of acyltransferases, which incorporate fatty acids into the *sn-1* and *sn-2* positions (19). With constant remodeling (incorporation,

release, re-incorporation), there appears to competition for the *sn-2* position among various PUFAs fatty acids, whereby LA and AA compete for incorporation (20).

Tissue phospholipids are responsive to dietary lipid intake following delivery of these lipids via the various circulating lipoproteins. Some tissue phospholipids respond more acutely to dietary PUFA (such as plasma phospholipids), while others exhibit more long term exposure, i.e., erythrocytes (21, 22). Erythrocytes are formed in the bone marrow with a life span of 120 days and the fatty acids found in these phospholipids represent a more stable pool of dietary PUFA compared to the plasma phospholipid pool (23, 24)

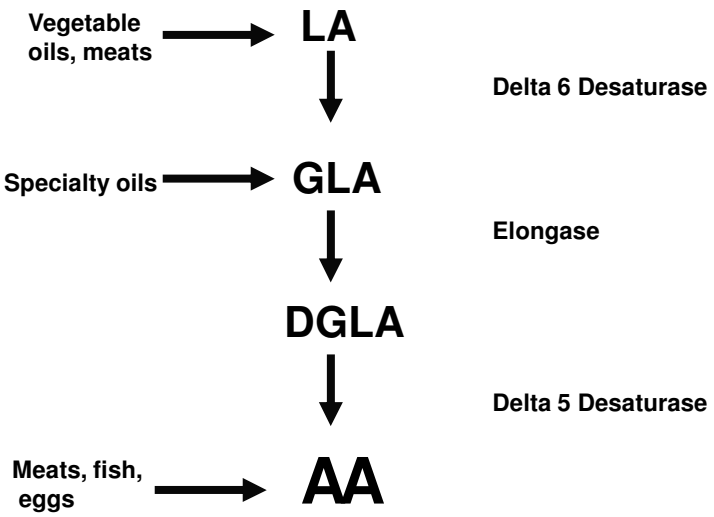

Figure 1. N-6 metabolic pathway. A series of enzymatic steps converts linoleic acid to highly unsaturated n-6 derivatives.

Arachidonic Acid Cascade

AA is a major PUFA associated with membrane phospholipids. When released from phospholipids via a variety of phospholipases, AA can be enzymatically oxidized to hundreds of bioactive derivatives called eicosanoids. The formation of these compounds are collectively referred to as the AA cascade and are mediated by a group of enzymes called cyclooxygenases (COXs) and lipoxygenases (LOXs), as well as enzymes associated with the cytochrome P450 system (Figure 2).

Eicosanoids

Eicosanoids are bioactive lipid compounds (*eicosa-* means 20) derived from highly unsaturated fatty acids with a length of 20 carbons, i.e. AA. The first eicosanoids were discovered from the seminal fluid of the prostate gland with smooth cell muscle relaxing properties and were termed “prostaglandins” (25-28). Subsequent research demonstrated that there were many prostaglandin-like compounds (29-31). In 1964, it was discovered that AA was the parent compound of these prostaglandins (32).

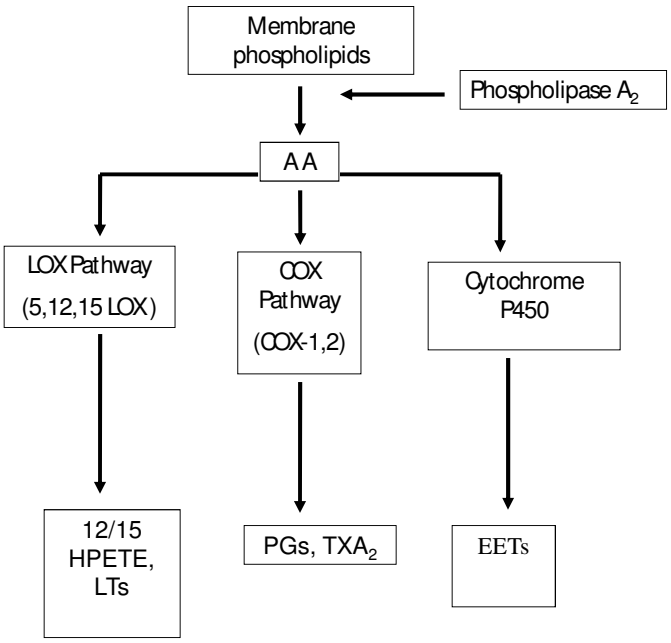

Figure 2. Arachidonic acid cascade. AA is converted to eicosanoids through lipoxygenase (LOX), cyclooxygenase (COX), and cytochrome P450 pathways.

Cyclooxygenase pathway

Prostaglandins are produced by the action of cyclooxygenase (COX) and are characterized by the addition of two molecules of oxygen forming a distinct endoperoxide ring. There are two isoforms, COX-1 and COX-2. COX-1 is constitutively expressed at relatively low levels in all tissues, and in some tissues is the only isoform, i.e., platelets. It has been identified to be important in a number of tissues. For example, it is involved in maintaining the integrity of the gastric mucosa (33), modulation of renal blood flow (34), and regulation of platelet aggregation (35). In contrast, COX-2 is the inducible isoform. It is expressed following stimulation of growth factors and proinflammatory cytokines, and as such, is the target of a number of selective COX-2 inhibitors such as Celebrex® (36). COX-2 is involved in

inflammation, cancer and cardiovascular disease (CVD) and increases in response to injury. The COXs are the committed steps for the conversion of AA to prostaglandin (PG)H_2, the parent compound to the 2-series prostaglandins (i.e., PGE_2), thromboxane (TXA_2) and prostacyclin (PGI_2) (37).

The LOXs are a family of lipid oxygenases that incorporate molecular oxygen on AA at specific sites forming lipid peroxide derivatives (38). If the oxygen is attached at carbon 5, it is referred to as a 5-LOX. If it incorporates oxygen at carbon 12, it is a 12-LOX, carbon 15, a 15-LOX, and so. The LOX pathways produce a variety of proinflammatory eicosanoids (39). 5-LOX is responsible for the formation of leukotrienes (LTs), such as LTB_4, LTC_4, LTD_4, LTE_4 (40). They are potent inflammatory mediators produced by a variety of tissues. LTB_4 is a key LT produced by leukocytes that is involved in activation of neutrophils (41), while the cysteinyl leukotrienes (LTC_4, LTD_4, LTE_4) are key mediators of asthma and anaphylaxis. Even vascular tissue, such as the aorta produces LOX products that along with leukocytes are believed to be critical for the progression of cardiovascular disease (42, 43)

1.6 Inflammation

Inflammation is a biological response to injury. Immune cells contribute to this response in a variety of ways, among them through the production of bioactive eicosanoids, such as PGs, LTs and TXA_2 and are a target for anti-inflammatory therapies, such as non-steroidal anti-inflammatory drugs (NSAIDs) (i.e., aspirin, Celebrex). Chronic and over-production of eicosanoids have been linked to arthritis, inflammatory bowel disease, atherosclerosis, chronic hepatitis, liver cirrhosis, asthma and psoriasis (44-48). Arachidonic acid is of interest because it is the precursor for the inflammatory eicosanoids and a number of recent reviews have outlined the role of dietary lipids and eicosanoids in inflammation and the immune response (47-51).

These reviews highlight the importance of antagonizing AA metabolism as a targeted intervention for treatment. Since tissue AA content influences inflammation, LA is thought to influence inflammatory biomarkers by enriching the AA pool (48), and therefore, contribute to inflammation in tissues (52). However, the link between LA and inflammatory biomarkers has not been substantiated and increasing levels of LA may in fact reduce inflammation (53, 54) Therefore, there appears to be contradictory evidence regarding the link between LA and AA-mediated inflammation.

1.5 CVD

A variety of eicosanoids are implicated in the pathogenesis of CVD and involves platelets, endothelial cells, macrophages, erythrocytes, mast cells, neutrophils, granulocytes, lymphocytes and vascular smooth muscle cells (55, 56). Platelets are key initiators and promoters in this process (57) and tissue AA content is positively associated with TXA_2 production (a pro-aggregatory eicosanoid) and platelet aggregation (58, 59). When activated, platelets produce TXA_2 and in turn recruit and activate surrounding platelets, resulting in vasoconstriction, platelet vascular wall interaction, the release of cysteinyl leukotrienes, more TXA_2 production and a plethora of a molecules (i.e., chemokines) that activate and recruit monocytes, leukocytes and lymphocytes, key mediators of vascular inflammation (57, 60, 61). Inhibition of platelet vascular wall interaction reduces atherosclerotic lesion formation (62), and inhibition of platelet COX activity is believe to be an underlying mechanism for the anti-atherogenic effects of NSAIDs, such as aspirin (63).

Following recruitment of monocytes to the subendothelium, activation/transformation of macrophages generate reactive oxygen species, tissue factor procoagulants, proinflammatory cytokines and a variety of eicosanoids via the 5-, 15-LOX and COX-2 pathways (56, 64).

Increased expression of 15-LOX is believed to be involved in oxidation of LDL (56, 65). Enrichment of macrophages with AA significantly increases the production of eicosanoids in general (14, 66), promoting the atherogenic process.

These data linking AA metabolism and cardiovascular disease provides some concern as to the link between its precursor LA and risk. If dietary LA were to be converted to AA and further enrich tissues with AA, this could contribute to the disease process. However, a recent set of reviews suggest that LA may reduce risk of CVD (67, 68).

1.7 Cancer

Inflammation is an underlying mechanism linked to many cancers i.e. hepatitis, inflammatory diseases of the bowel (69-73), and bioactive lipids from the AA cascade are key mediators of inflammation. In particular, COX-2 and its downstream AA product, PGE_2, has singled out as a key cancer promoter (74). Wang and Dubois elegantly describe how COX-2 and PGE_2 in particular are involved in proliferation, immuno-suppression, angiogenesis, apoptosis and metastasis and invasion (75). Overexpression of COX-2 is a characteristic of many malignancies (76-78) and inhibition or down regulation of this enzyme attenuates tumorigenesis (79, 80). It is believed that PGE_2 is the key mediator in this process (81). PGE_2 induces intestinal epithelial cell proliferation and COX-2 expression in adenomas in mice with a defect in the *Apc* gene (mutation of an early gene involved in human colorectal cancer) (82). Similarly, expression of 15-prostaglandin dehydrogenase and the ability to degrade PGE_2 is reduced in tumors compared to normal tissue (78). Coupled with over expression of COX-2, enriching tissues with AA would augment PGE_2 levels and enhance its protumorigeneic activity. This was observed when $Apc^{Min/+}$ mice were treated with PGE_2 (83).

In support of these results, inhibition of the conversion of LA to prostaglandins, through AA, attenuates tumorigenesis (79, 84-86). This is particularly important from a diet perspective. Antagonism of AA metabolism (either with NSAIDs or long chain omega-3 fats) inhibits tumorigenesis, and by-passing these inhibitions restores tumor load. For example, the omega-3 fatty acid EPA may reduce tumor load by attenuating AA content, and therefore the AA cascade (85). Most importantly is that the AA cascade is restored with dietary AA, even when fed alongside EPA (87). Critically important to this discussion is the fact that selectively inhibiting delta-6 desaturase in mice fed a diet containing LA, reduced tumor number by 35%, while the concomitant addition of AA with the inhibitor had no effect as compared to controls (88). These data are consistent with the extensive literature outlining the protective effects of NSAIDs on colorectal cancer (89, 90).

1.8 Dietary Linoleic Acid and Tissue Arachidonic Acid

LA is the sole contributor to tissue AA in the absence of other dietary n-6 PUFA. This was first demonstrated in rodent studies. When rodents were fed lipid-deficient diets, a non-linear, dose-dependent enrichment of tissue AA occurred with increasing levels of dietary LA. In these studies, the diets were devoid of other PUFA, essential and non/conditionally-essential, that could contribute to the maintenance of physical properties required for normal cell function (i.e., membrane fluidity). In compensation, this could potentially exacerbate the rate and extent of this conversion. However, in the presence of other PUFA with similar properties, such as long chain n-3 PUFA, this relationship becomes more complicated in that they can be a suitable replacement for AA in tissues. Therefore, providing rodents with diets containing only LA may demonstrate that LA can and is metabolized to AA, eventually reaching a threshold (11), but the ability to quantitatively extrapolate these results to humans is uncertain.

In humans, LA is the major dietary PUFA in the Western diet at 12-17 grams per day for women and men, respectively (91), or approximately 6% of energy. The consumption of LA coincides with the commercialization and availability of vegetable oils (92). This intake is complemented by consumption of other PUFA where each PUFA family competes for metabolism and incorporation into membrane phospholipids. If n-6 PUFA is the predominant family, then the fatty acids from this family should theoretically dominate membrane phospholipids.

The minimum requirement for dietary LA has been estimated to be less than 2% of energy in humans (13, 93), and the levels of LA in the diet to achieve essentiality in infants could be as low as 0.5% of energy (13, 94). In adults, it has been reported that tissue levels of AA no longer change in response to increasing dietary amounts of LA above 2% energy (95). However, others report changes at higher intakes of LA (96). As a result, these n-6 rich diets conjure up unwanted scenarios of dose-dependent enrichment of tissues with AA and subsequent chronic and over-production of bioactive eicosanoids (97, 98). Proponents of this view recommend reducing LA levels (97, 99); however, there is little clarity as to what those levels should be. Some recommend consumption of LA be limited to less than 2% of energy (100), while other suggest any reductions can be beneficial (97, 98, 101).

The recommendation of limiting LA to less than 2% was most likely based on the evidence that AA content in neutrophils was correlated with LA only when those intakes were below 2% of energy, where higher intakes had no effect (95). Similar evidence was shown in plasma cholesterol esters where AA content was unaffected by increasing intakes of LA at levels greater than 2% (102). Furthermore, a number of studies reported inverse relationships with

dietary LA and changes in tissue AA content (103, 104).With these discrepancies, controversy exists as to whether dietary LA does influence tissue AA content.

In summary, there is little dispute in the literature that when LA intake is consumed at levels less than 2% energy there may be a positive relationship between dietary intake and changes in tissue AA content. However, will this relationship be observed when those levels are above 2% energy? Furthermore, since the current levels of dietary LA has been estimated to be approximately 6% of energy, and this essential fatty acid is typically consumed within a matrix of other PUFA, should the general public be concerned?

With the conflicting evidence, compounded by pundits who proclaim an unmistakable link between dietary LA and tissue AA levels, the controversy regarding this issue continues. Recent review articles state that "the higher concentrations of LA typically found in the Western diet results in a greater conversion of LA to arachidonic acid" (105). Therefore as a result, "excessive n-6 precursors promotes formation of AA...high intakes of n-6 PUFA may contribute to development of neuroinflammation" (106). Even more pronounced statements describe the potential consequences of increased LA in the Western diet:

> Because of the increased amounts of omega-6 fatty acids in the Western diet, the eicosanoid metabolic products from AA, specifically prostaglandins, thromboxanes, leukotrienes, hydroxy fatty acids, and lipoxins, are formed in larger quantities… Thus, a diet rich in omega-6 fatty acids shifts the physiological state to one that is prothrombotic and proaggregatory, with increases in blood viscosity, vasospasm, and vasoconstriction and decreases in bleeding time..; (101).

In summary, pundits proclaim the detrimental impact of increase dietary LA on tissue AA, and therefore chronic diseases. Interestingly, no one has systematically reviewed the relationship between dietary LA and changes in tissue AA content, particularly within the context of a Western diet.

1.9 Research Objective

The objective of this study was to explore the relationship between dietary LA and changes in tissue AA content within the context of a Western-type diet. This objective will be accomplished via the following specific aims:

1. To determine if changes in LA consumption are correlated with changes in AA content of phospholipids in plasma/serum and erythrocytes in studies where subjects are consuming, for the most part, diets reflective of those in the general population.

2. If dietary LA does not modify tissue AA levels, determine if that effect is the result of tissue saturation of AA or inhibition of conversion of LA to AA.

Chapter 2

Increasing Dietary Linoleic Acid Does Not Increase Tissue Arachidonic Acid Content in Adults Consuming Western-Type Diets

ABSTRACT

Background: Linoleic acid, with a DRI of 12-17g/d, is the most highly consumed polyunsaturated fatty acid in the Western diet and is found in virtually all commonly consumed foods. The concern with dietary linoleic acid, being the metabolic precursor of arachidonic acid, is its consumption may enrich tissues with arachidonic acid and contribute to chronic and overproduction of bioactive eicosanoids. However, no systematic review of human trials regarding linoleic acid consumption and subsequent changes in tissue levels of arachidonic acid has been undertaken. **Objective:** In this study, we reviewed the human literature that reported changes in dietary linoleic acid and its subsequent impact on changing tissue arachidonic acid in erythrocytes and plasma/serum phospholipids. **Design:** We identified, reviewed, and evaluated all peer-reviewed published literature presenting data outlining changes in dietary linoleic acid in adult human clinical trials that reported changes in phospholipid fatty acid composition (specifically arachidonic acid) in plasma/serum and erythrocytes within the parameters of our inclusion/exclusion criteria. **Results:** Decreasing dietary linoleic acid up to 90% was not significantly correlated with changes in tissue arachidonic acid levels ($p=0.39$). Similarly, when dietary linoleic acid levels were increased six fold, no significant correlations with tissue arachidonic acid levels were observed ($p=0.72$). However, there was a positive relationship between dietary gamma-linolenic acid and arachidonic acid on changes in tissue arachidonic levels. **Conclusions:** Our results do not support the concept that modifying current intakes of dietary linoleic acid has an effect on changing tissue levels of arachidonic acid in adults consuming Western-type diets.

Introduction

Arachidonic acid (AA) is a potent bioactive molecule. When released from membrane phospholipids, it is converted to a variety of bioactive compounds, called eicosanoids. These oxidized lipid molecules are related to a number of chronic diseases including cardiovascular disease, cancer and inflammation (48, 75, 107, 108). Enrichment of AA in tissues is positively correlated with the production of eicosanoids. Linoleic acid (LA) is the major dietary polyunsaturated fatty acid (PUFA) in the Western diet and is a metabolic precursor to AA, linked biochemically via two desaturases and an elongase. Typical intakes of LA are 12-17 grams per day for women and men, respectively (91), or approximately 6% of energy. In the absence of other n-6 PUFA (including dietary AA), dietary LA is the sole contributor to tissue AA. This relationship had been established in experimental rodent models where dietary LA was correlated with tissue AA content in a non-linear relationship in rats provided fat-free background diets (109) and lipid-rich diets (110).

Recent reviews suggest this relationship may exist in adult humans consuming a typical Western-type diet (105, 106) and some have recommended limiting LA intake as a way to help reduce tissue AA levels (97, 101). Certainly, this relationship had been reported in subjects consuming diets containing LA at levels less than 2% of energy (95). There are, however, a number of recent papers suggesting that increasing dietary LA does not increase tissue AA levels, but in fact may have an inverse relationship (111, 112). To compound the complexity of this relationship, the family of n-6 PUFA are, in general, synonymously identified to dietary LA, while seemingly ignoring other members who can contribute to tissue AA, i.e., dietary gamma-linolenic acid (GLA) and AA.

This study was designed to explore the relationship of dietary LA and tissue AA. This is the first study to review the literature as to whether increasing dietary LA is positively correlated with increasing tissue AA content, and whether reducing dietary LA has the opposite effect in adults consuming Western-type diets. We further investigated what potential contributions other dietary n-6 PUFA may have on tissue AA content. This study was limited in scope and did not address other controversial issues related to dietary PUFA or their health effects.

Methods

The aim of this paper was to identify, review, and evaluate all peer-reviewed published literature presenting data outlining changes in dietary LA in adult human clinical trials which report changes in phospholipid fatty acid composition (specifically AA) in plasma/serum and erythrocytes. Further refinements to the search strategy included reported changes in tissue AA levels following changes in dietary intake of AA and its various n-6 PUFA precursors, i.e., LA and GLA. Published articles meeting eligibility criteria from 1970 to present were reviewed, of which 4336 articles were retrieved from May 2009 - November 2009. The primary search engine used was PubMed.gov (The National Library of Medicine, National Institutes of Health), along with several prominent nutrition-based clinical journals, i.e., American Journal of Clinical Nutrition, British Journal of Nutrition, and any additional citations in articles reviewed. The search terms included linoleic acid, γ-linolenic acid, gamma-linolenic acid, arachidonic acid, omega-6, n-6, olive oil, soybean oil, sunflower oil, safflower oil, corn oil, omega-3, n-3, plasma, erythrocyte, red blood cell and phospholipid. After an initial review of the papers, 4043 were excluded because of insufficient data or studies that did not investigate our parameters. Of the 293 papers that passed the initial review process, each was reviewed by two independent investigators (BR and JW) and thirty-six were acceptable by both reviewers. Those papers that

were not accepted (n=249) were rejected because baseline data was not sufficiently reported, data for target tissues was not presented, insufficient data was present and did not allow for appropriate calculations, background diets were not sufficiently described, or they included supplementation of restricted food items (i.e., long chain n-3 PUFA).

The following eligibility criteria applied to all accepted articles. Subjects had to be 18 years or older with no known metabolic disorder that would influence tissue AA content. Sufficient data on LA, GLA and/or AA consumption (pre- and post-intervention) was required. The nature of the intervention (i.e., capsules, oils or dietary modifications) had to be presented. The fatty acid data (plasma/serum and/or erythrocyte) had to be determined from fasting patients, pre- and post-supplementation. Baseline and post-treatment tissue phospholipid fatty acid composition had to be provided. On occasion, percent changes in tissue fatty acid composition were provided and this data was used. Only those papers published after 1970 due to improved gas chromatographic methods were accepted. Articles were automatically excluded if subjects were less than 18 years old, pregnant or nursing, consuming supplements containing long chain n-3 fatty acids or supplemented fish intake above and beyond their typical dietary regimen, or using known inhibitors of AA metabolism, such as non-steroidal anti-inflammatory drugs (NSAIDs). Thirty-six articles were found to meet all of the inclusion-exclusion criteria.

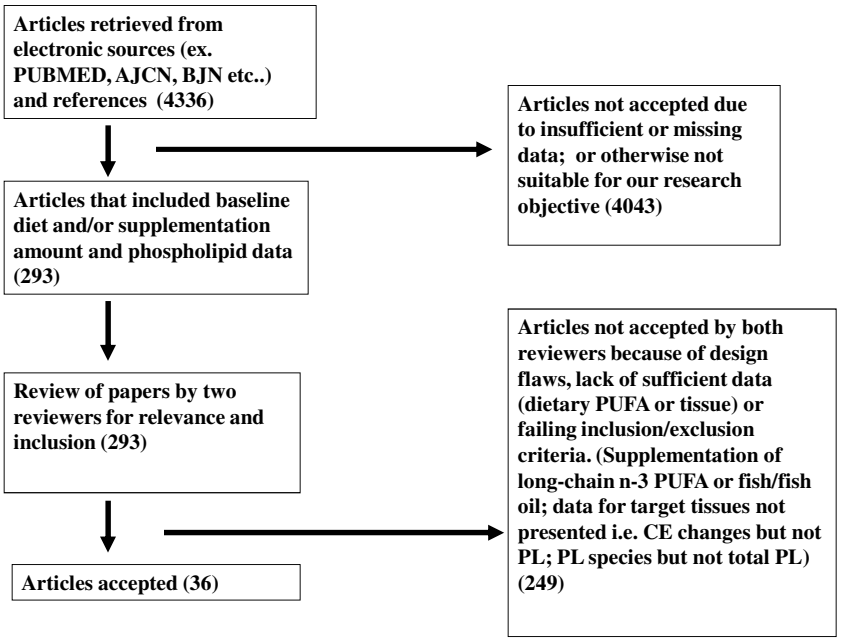

Figure 3. Schematic outlining the systematic review

Once accepted, data on dietary n-6 fatty acid intake (% of calories or g/d) and tissue AA content were extracted. Study design, number and gender of subjects, method of supplementation (i.e., type of oil, capsules or food component used) were recorded. Those studies involving dietary LA as percentage of calories or g/d and its effects on changes in tissue AA content are summarized in Tables 1-3. Similarly, those studies involving dietary GLA (mg/d) and AA (mg/d) are summarized in Tables 4 and 5. If a study met the eligibility criteria and contained more than one comparison, each comparison was reported as a separate data point.

Baseline tissue AA levels were defined as relative abundance of AA in tissue phospholipids prior to dietary supplementation (or reduction) of the corresponding dietary n-6 PUFA of interest (i.e., LA, GLA or AA). In the case of cross-over designs, baseline fatty acid composition was established following a washout period or after supplementation of a control diet if there were no or only minor changes in the dietary n-6 PUFA content. For example, a

supplement rich in oleic acid (a monounsaturated fatty acid typically used as a control and known to have a neutral effect on tissue AA content) could be used as a control lipid (or oil) prior to supplementation of an equal amount of a lipid (or oil) rich in LA. Percent change for each dietary n-6 fatty acid of interest was used to standardize the relative differences between baseline intakes and intervention intakes following the experimental period using the following formula:

$$\frac{\text{intervention intake - baseline intake}}{\text{baseline intake}} \times 100$$

The levels of intake were based on the relative caloric amount (% of calories), and when this data was not available absolute intake levels (mg/g or g/d) were used. Percent change for tissue AA content was used to standardize the relative differences between baseline levels and intervention levels following the experimental period using the following formula:

$$\frac{\text{post-intervention content - baseline content}}{\text{baseline content}} \times 100$$

Statistical Analysis

The overall linear correlation between percent change of dietary n-6 fatty acids and percent change of tissue AA was computed using the Proc Corr procedure in SAS 9.2 (SAS Institute Inc. SAS Campus Drive, Cary, North Carolina). The correlation matrix and the T statistic tested for correlation and statistical significance, respectively. For the linear correlations, the equation of the line was computed, and represented in y=mx+b for those that exhibited linearity. Data not resembling a linear relationship (i.e. dietary GLA and AA) utilized a polynomial growth curve from SAS General Linear Model and t-tests for model parameters tested for significance. P-values less than or equal to 0.05 were considered significant. The Y

values represent changes of AA (% from baseline) and the X values represent the changes of the various dietary n-6 PUFA (% from baseline or mg/g supplemented). In addition to the overall correlation test, the statistical significance for each individual data point (for changes in tissue AA), as reported by the authors in their respective manuscripts, was identified in each graph. If the changes from baseline were significantly different the data was represented by triangle. If the changes from baseline were not statistically different, they were represented by a diamond.

Results

Twelve comparisons reported decreases in LA intakes (-12% to -90%) and no significant correlations were associated with changes in plasma/serum phospholipid AA content *(*r^2=0.05, p=0.44, y= (-0.090*x)-6.56) (**Figure. 4** and **Table 1**). Only one study of the twelve reported a significant change, a 4.1% increase in AA content, following a 29% reduction in LA intake (113).

Increases in dietary LA, ranging from 12%-550%, were not significantly correlated with changes in plasma/serum phospholipid AA content (r^2=0.03, p =0.45, y= (-0.002*x)-0.09) (**Figure. 5** and **Table 1**). Of the seventeen comparisons, only four studies reported significant changes in AA levels when dietary LA levels were increased; three studies reported 3-20% reductions following 12%-110% increases in LA consumption (103, 113, 114) and only one study reported a significant increase in AA content (10%) following an 86% increase in LA intake (114). Sub-dividing the studies by design (crossover versus non-crossover) had no effect on the results (data not shown). Similarly, in those studies that only reported absolute levels of LA supplementation (g/d), increasing LA supplementation was not significantly correlated with changes in plasma/serum phospholipid AA content (r^2=0.06, p=0.64, y=(0.793*x)+1.17) (**Figure. 6** and **Table 2**). Of the fourteen comparisons, only two were significantly different, one

resulted in an increase in AA content by 3% following supplementation of 2.24 g/d of LA (115) and the other resulted in a reduction of AA content by 7% following supplementation of 0.86 g/d (116).

Similar comparisons were made in erythrocytes with increasing and decreasing intakes of LA, although the number of studies were more limited. Increases in dietary LA, ranging from 12%-100%, were not significantly correlated with changes in tissue AA content *(*r^2=0.06, p=0.75, y = (- 0.133*x)-1.24) (**Figure. 7**). Reducing dietary LA intake (-12% to -70%) was not significantly correlated with changes in tissue AA content (r^2=0.02, p=0.77, y = (-0.014*x)+0.17) (**Figure. 8**). In addition, out of the seven studies, only one study reported a significant change where decreasing dietary LA intake by 29% resulted in a 4% increase in AA content (113).

Seven studies met our criteria for the effects of GLA supplementation on changes in plasma/serum phospholipid AA content (**Figure. 9**). There appeared to be a dose dependent increase in AA content with increasing intakes of GLA (ranging from 360-6000 mg/day). This positive correlation (r^2=0.75, p=0.03, y= (0.004*x)+7.36) was significant with a linear regression model, but not with a non-linear (polynomial) regression model *(p=0.19)*. Of the six AA data points, four of them reported statistically significant increases.

Similiarly, increasing dietary AA (0.5 g/d to 6 g/day) was positively correlated with increases in plasma/serum phospholipid AA content using a linear regression model (r^2=0.83, p=0.02, y= (0.018*x)+38.60) (**Figure. 10**), but not with a non-linear (polynomial) regression model (*p=0.16*). All data points were reported as significantly different (**Table 4**).

Table 1. Studies outlining the effects of decreasing dietary linoleic acid levels (% energy) from baseline on changes in plasma/serum phospholipid arachidonic acid content

Author, (reference)	Study design	Subjects	Diet length	Diet comparison	LA (% change) Baseline to intervention	ΔAA (%) change	Comments
Lichtenstein (117)	Randomized double-blind crossover	N=30	35 days	Soybean oil diet (baseline) compared to high oleic acid soybean oil diet	-82 (11%-1.9%)	N.S.	Pooled data of men and women. Baseline diet of 10.96% energy closest to DRI for LA. AA did not differ among remaining groups tested either
Lasserre (103)	Randomized crossover	N=24	5 months	Peanut oil diet (baseline) compared to milk fat diet Peanut oil diet (baseline) compared to low erucic acid rapeseed oil (canola oil) diet	-90 (6.5%-0.6%) -30 (6.5%-4.5%)	N.S. N.S.	Used peanut oil group b/c close to DRI for LA. Subjects were nuns in monastery. Used peanut oil group b/c close to DRI for LA. Subjects were nuns in monastery.
Liou (118)	Randomized crossover	N=24	4 weeks	High linoleic sunflower oil (diet) compared to high oleic acid safflower oil (diet)	-63 (10.5%-3.8%)	N.S.	Incorporated test oils into baked foods (cookies, breads), mayonnaise, salad dressing. AA data presented in graphs, not tables. Fish intake was avoided for all groups. AA PL content did not differ between sequence of diets going from high LA to low LA or vice versa. Study address low or high LA with constant ALA at 1%
Vega-Lopez (119)	Randomized crossover	N=15	35 days	Canola oil diet compared to palm oil diet	-50 (6.5%-3.3%)	N.S.	Canola oil in mixed foods was replaced by palm oil in mixed foods. AA did not change among all three dietary groups. Canola oil diet is baseline because closest to DRI LA intake.
Geppert (120)	Randomized double- blind intervention	N=54	8 weeks	Baseline diet compared to LA reduced diet (using olive oil capsules)	-12 (5.8%-5.1%)	N.S.	Used olive oil capsules with vegetarians.
King (113)	Randomized parallel	N=66	6 weeks	Baseline diet compared to low fat diet.	-29 (10%-7.1%)	+4.1[a]	Used modified food items for diets containing different amounts of fat. Reported AA PL in % change.
Li (122)	Parallel intervention	N=17	28 days	High LA diet to moderate LA diet using canola oil/canola margarine	-48 (13.5%-7%)	N.S	Subjects were given diet more than twice DRI for LA and then given diet resembling the DRI for LA. All groups were asked to not consume fish.

				High LA diet to normal LA diet using canola oil/canola margarine	-39 (11.9%-7.3%)	N.S.	
Mantzioris (123)	Parallel intervention	N=15	4 weeks	Control diet (sunflower oil) group compared to intervention diet (flaxseed oil)	-57 (7.8%-3.3%)	N.S.	Control group consumed relatively close to DRI for LA while intervention group reduced LA by more than half.
Goyens (121)	Double-blind intervention	N=19	6 weeks	Reduced LA in food items (margarines, pastries, baked goods)	-57 (7%-3%)	N.S.	Test oils consumed in margarine and pastries. Prohibited consumption of fish or marine foods in all groups.

[a]Percent change (±) from baseline in AA that is significant ($p<0.05$). Non-significance is denoted by N.S.

Abbreviations: AA, arachidonic acid; DRI, Dietary Reference Intake; LA, linoleic acid

Table 2. Studies outlining the effects of supplementing dietary linoleic acid levels (g/day) on changes in plasma/serum phospholipid arachidonic acid content.

Author, (reference)	**Study design**	Subjects	**Diet length**	**Diet comparison**	**LA (% change) Baseline to intervention**	**Δ AA (%) change**	**Comments**
Thijssen (124)	Randomized multiple crossover	N=45	5 weeks	Stearic acid diet to oleic acid diet in food items (using margarines, breads, sponge cakes) Oleic Acid to Linoleic Acid food items (margarines, breads, sponge cakes)	14 (2.1% -2.4%) 287 (2.4%-9.3%)	N.S. N.S.	No order to diets given. Subjects received all 3 diets with varying amounts of LA.
Lichtenstein (117)	Randomized double-blind crossover	N=30	35 days	Baseline soybean oil diet compared to Low saturated fat-soybean oil diet. The soybean oils varied in linoleic acid composition Baseline high oleic-soybean oil diet to low ALA-soybean oil diet	15.8 (11% -12.7%) 552 (1.9 %-2.5%)	N.S. N.S.	Pooled data of men and women. Five diets of random order. Subjects visited metabolic kitchen 3 times week. Varying LA amounts using modified soybean oils.
Vega-Lopez (119)	Randomized crossover	N=15	35 days	Canola oil diet compared to soybean oil diet	64.2 (6.5%-10.7%)	N.S.	Canola oil in mixed foods was replaced by soybean oil in mixed foods. AA did not change among all three dietary groups. Canola oil diet is baseline because close to the DRI for LA.

Liou (118)	Randomized crossover	N=22	4 weeks	Low LA diet (high in oleic acid safflower oil) compared to high LA diet (high in sunflower oil)	176 (3.8%-10.5%)	N.S.	AA PL content presented as graphs, not numerically. Fish was avoided in all dietary groups. ALA intake was kept constant between low/high diet
Valsta (96)	Randomized crossover	N=39	6 weeks	Habitual diet compared to high linoleic acid trisunflower oil diet.	86 (4.2%-7.8%)	+10	Fish cut in half in all dietary groups. Has a baseline for before each diet. Used trisunflower oil in margarine, food oil, salad dressing, bread, cake and cookies, in place of habitual foods.
Raatz (114)	Randomized crossover	N=10	28 days	Low fat diet (20% energy) compared to high fat diet (45% energy).	100 (6%-12%)	-16	Modified foods rich in linoleic acid. Random order to diet, so baseline was chosen based on DRI of LA. Used washout period of 21-28 days
Lasserre (103)	Randomized crossover	N=24	5 months	Peanut oil diet compared to sunflower oil	111 (6.5%-13.7%)	-20	Used peanut oil group b/c close to DRI for LA.
Innis (111)	Randomized crossover	N=24	8 weeks	Low LA diet to high LA diet	176 (3.8%-10.5%)	N.S.	Controlled for dietary AA.
King (113)	Randomized parallel intervention	N=33	6 weeks	Baseline diet compared to moderate fat diet.	13 (10.3%-1.6%)	-3.2[a]	Used modified food items for diets containing different amounts of fat. Reported AA PL as % change.

Li (122)	Parallel intervention	N=10	14 days	Baseline Western diet to intervention diet increased in LA intake using safflower oil	17.8 (10.1%-1.9%)	N.S.	Used safflower oil/safflower margarine to increase LA in diet to almost twice DRI of LA. AA did not differ among all groups in study.
		N=7	14 days	Baseline Western diet to intervention diet increased in LA intake using safflower oil	82.4 (7.4%-13.5%)	N.S.	
Montoya (125)	Sequential interventions	N=41	4 weeks	From palm oil based diet compared to olive oil based diet.	16 (3.2%-3.7%)	N.S.	Used nuns and priests. Everyone consumed same sequence of diets. AA did not change among the three test diets. No crossover, subjects were their own controls.
				Olive oil based diet to sunflower oil base diet	229.8 (3.7%-12.2%)	N.S.	

[a]Percent change (±) from baseline in AA that is significant ($p<0.05$). Non-significance is denoted by N.S.

Abbreviations: AA, arachidonic acid; DRI, Dietary Reference Intake; LA, linoleic acid

Table 3. Studies outlining the effects of supplementing dietary linoleic acid levels (g/day) on changes in plasma/serum phospholipid arachidonic acid content.

Author, (reference)	Study design	Subjects	Diet length	LA source, amount supplemented (g/d)	Δ AA (%) change	Comments
Thies (127)	Randomized, double-blind, parallel intervention	N=8	12 weeks	Oil blend (0.64) Placebo oil (0.9)	N.S. N.S.	Different oil blends were sources of LA
Geppert (116)	Randomized double- blind parallel intervention	N=20	8 weeks	Oil blend (0.86)	-7[a]	Blend palm, rapeseed and sunflower oil
Johansson (128)	Randomized, double-blind, crossover	N=12	4 weeks	Sea buckthorn berry oil (0.90)	N.S.	Sea buckthorn berry oil is 17.9% LA
Kew (129)	Double-blind, parallel intervention	N=42	4 weeks	Olive oil (0.92)	N.S.	
Buckley (130)	Double-blind parallel intervention	N=45	4 weeks	Olive oil (0.95)	N.S.	
Yaqoob (131)	Randomized, double- blind parallel intervention	N=8 per group	12 weeks	Placebo (coconut/soybean oil) (1.0) Olive oil (1.2) Sunflower oil (6.95)	N.S. N.S. N.S.	
Wallace (132)	Randomized, double- blind parallel intervention	N=8 N=8	12 weeks	Oil blend (1.52) palm/soybean oil (1.7)	N.S. N.S.	
Miles (133)	Randomized, double-blind parallel intervention	N=8	12 weeks	Placebo (palm/sunflower oil) (2.07)	N.S.	
Grimsgaard (115)	Double-blind, parallel intervention	n=78	7weeks	Corn oil (2.24)	+3.1	

Conquer (134)	Double-blind, parallel intervention	N=24	42 days	Corn oil (2.39)	N.S.	
Finnegan (135)	Double-blind, parallel intervention	N=50	6 months	Safflower/sunflower (11.6)	N.S.	Test oils provided as margarine and capsules
Anderson (126)	Parallel intervention	N=8 N=9	3 months	Olive oil (0.2) Olive oil (0.2)	N.S. N.S.	Olive oil supplement Provided LA for two different groups

Table 4. Studies outlining the effects of supplementing dietary gamma-linolenic acid on changes in plasma/serum phospholipid arachidonic acid content.

Author, (reference)	Study design	Subjects	Diet length	GLA source, amount supplemented (mg/d)	Δ AA (%) change	Comments
Thavonen (126)	Randomized, double-blind crossover	N=15	3 weeks	Black current seed oil (378)	N.S.	Subjects aged 55-75 years old.
Mills (127)	Randomized double-blind parallel intervention	N=10	28 days	Borage oil (1300)	+12	AA data available for only pre and post intervention (28 days).
Miles (128)	Randomized double-blind intervention	N=8-12	12 weeks	Borage oil capsules (2000)	+15	Consumed capsules for 12 weeks. AA only increased after the 8th week, no difference after 8th week.
Theis (129)	Double-blind parallel interventions	N=8	12 weeks	GLA-rich triacylglycerol capsules (770)	+27[a]	Subjects consumed capsules for 12 weeks. AA changed only on 12th week.
Yaqoob (130)	Double-blind parallel intervention	N=8	12 weeks	Evening primrose oil (1062)	N.S.	
Ebden (131)	Double-blind intervention	N=6	8 weeks	Efamol oil (360)	N.S.	No crossover with placebo. Subjects were asthmatics used medication or bronchodilator.
Johnson (132)	Pre-post intervention	N=5	3 weeks	Ultra-GLA capsules (6000)	+31	

[a]Percent change (±) from baseline in AA that is significant ($p<0.05$). Non-significance is denoted by N.S.

Abbreviations: AA, arachidonic acid; GLA, gamma-linolenic acid

Table 5. Studies outlining the effects of supplementing dietary arachidonic acid on changes in plasma/serum phospholipid arachidonic acid content.

Author, (reference)	Study design	Subjects	Diet length	AA source, amount supplemented (mg/d)	Δ AA (%) change	Comments
Ishikura (133)	Double-blind crossover	N=25	1 month	SUNTGA40S capsules (720)	+27	Derived from Mortierella alpina
Nelson (134)	Single blind crossover intervention	N=10	50 days	ARASCO (1490)	+85	Had 65 day washout period. Derived from Mortierella alpina
Theis (129)	Randomized, double-blind, parallel intervention	N=48	12 weeks	ARASCO (680)	+85	Derived from Mortierella alpina
Theis (129)	Randomized, double-blind, parallel intervention	N=48	12 weeks	ARASCO (680)	+85	Derived from Mortierella alpina
Kusmoto (135)	Double-blind intervention	N=12	4 weeks	SUNTGA40S (838)	+45	Derived from Mortierella alpina
Sinclair (136)	Parallel intervention	N=4	7 days	White meat/eggs	+52[a]	Consumed AA rich, low fat diet.
Seyberth (59)	Single blind intervention	N=4	2-3 weeks, depending upon subject	Capsules, AA ethyl ester (6000)	+136	Averaged from all 4 subjects.

[a]Percent change (±) from baseline in AA that is significant ($p<0.05$). Non-significance is denoted by N.S.

Abbreviations: AA, arachidonic acid

2. Discussion

Arachidonic acid is arguably the most important PUFA associated with membrane phospholipids. Upon release, AA can be enzymatically metabolized to a surfeit of bioactive derivatives, eicosanoids, known to contribute to a variety of chronic diseases (48, 75, 107, 108). The relative abundance of AA in membrane phospholipids positively influences eicosanoid production (137). It is well known that dietary PUFA can affect tissue AA levels; however, what is uncertain and wrought with controversy is whether modifying current intakes of dietary LA will result in concomitant changes in tissue AA content. Therefore, if the public chose to consume less LA, would tissue AA levels go down, and antithetically, if the public consumed more LA would tissue levels of AA go up? The goal of this paper was to ascertain the relationship between dietary LA and tissue AA content in adults consuming a Western-style background diet. It was not designed to address other controversies surrounding the issues of dietary PUFA or in other population groups.

Many papers interchange the more general term n-6 PUFA for dietary LA, but there are two major n-6 PUFA, LA and AA, and they are distributed unevenly in the Western diet. While LA is the major PUFA in almost every commonly consumed food, AA is exclusively found in animal products, such as, muscle, organ meats and eggs (138). They have distinct biological activities that are biochemically linked via desatuation and elongation, and as such, LA is the conditionally essential fatty acid. Linoleic acid is specifically required in the skin to maintain the integrity of the epidermal water barrier and AA is the immediate precursor to eicosanoids, as well as being the n-6 PUFA selectively incorporated into the membranes of certain tissues, i.e., brain (139). When consumed (LA vs. AA) they appear to have differential effects on tissue fatty

acid composition, where AA appears to more robustly modify tissue AA levels and eicosanoids (112, 140).

The data presented in this paper does not suggest that a dose response between dietary LA and tissue AA exists within the backdrop of individuals consuming a Western-type diet. Increasing LA by up to 551% and reducing LA by as much as 90% failed to yield compelling evidence supporting the concept that any conversion of dietary LA to downstream metabolites results in tissue enrichment of AA, a notion commonly asserted (105). We chose to evaluate the data by looking at changes from baseline in tissue AA content to standardize the data from one study to the next. Each study began with a baseline value and we reported percent changes from that baseline. Supplemental intakes of LA were reported based on energy and when that value could not be determined, we reported absolute supplemented values, and these data were reported separately.

As observed from the distribution of the responses, there was wide variability. Some papers showed small increases in tissue AA levels when dietary LA changed, while other papers showed small decreases, but most of these changes lacked significance. When there was significance, the changes were minimal and the distribution pattern of the data did not favor an increase or a decrease. We chose plasma/serum and erythrocytes as the tissues of choice because here is where the plethora of data exists in the human literature. Erythrocytes are a more stable pool of dietary lipids, contain very little neutral lipids and thus represents a membrane fraction of AA. Fasting plasma/serum phospholipid levels primarily (but not exclusively) represents the membranes of lipoproteins derived from the surface of hepatic endoplasmic reticulum, and this pool is more responsive to more recent dietary PUFA intakes.

In an effort to identify why dietary LA may not modify tissue AA levels, we reviewed the literature for dietary GLA using the same search strategy. Was the conversion of LA to AA rate-limiting, or were tissue levels of AA saturated? Delta-6 desaturase is the rate-limiting enzyme in the metabolism of LA to AA. GLA is a dietary n-6 PUFA that enters the metabolic pathway after the delta-6 desaturase step. If delta-6 desaturase is rate-limiting and tissue AA content is not saturated, then there should be evidence that including GLA in the diet increases tissue AA levels. When GLA was supplemented as the triacylglycerol form or as a component of a dietary oil containing GLA (i.e., blackcurrant, evening primrose or borage oil), tissue AA content increased in a dose responsive manner. These effects appeared to be less prominent in those studies (126-128) that used oils containing appreciable amounts of the more highly unsaturated n-3 PUFA stearidonic acid, i.e., blackcurrant or borage oil (138). When AA was supplemented in the diet, there was further enrichment in tissue AA content above that observed with either LA or GLA. These results suggest that delta-5 desaturase potentially becomes rate limiting when GLA is supplemented. The reaction mediated by delta-5 desaturase is an intermediate step between GLA and AA and by-passing that step with dietary AA leads to further enrichment. These data seem to suggest that while dietary LA maybe a metabolic precursor for AA, its influence on tissue levels are limited by the enzymatic conversion through delta-6 desaturase and not due to tissue saturation of AA. This data is supported by the poor rates of conversion of plasma/serum LA to AA in adults. In tracer studies involving stable isotopes, the estimated fractional conversion of LA to AA was between 0.3% and 0.6% (141).

The levels of LA in the diet to achieve essentiality could be as low as 0.5-2.0% of energy in infants (13, 94) and it has been reported that tissue levels of AA no longer respond to dietary LA intakes above 2% energy in adults (95). Our study was designed to choose studies that

incorporated a Western-type diet where LA is not typically limiting, reflective of the general public. This means a full complement of PUFAs were being consumed along with LA supplementation. The DRIs for LA and ALA are 12g-17g/d and 1.1g-1.6g, respectively (women the lower figure, men the higher figure). This would be equivalent to intakes approximating 6% and 0.7% of calories per day for LA and ALA, respectively. It is not unreasonable to think that with a background diet containing LA, ALA, AA, and long-chain n-3 PUFAs, i.e. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at typical intakes, that modifying LA levels may not influence tissue AA levels.

A few studies specifically evaluated the impact of dietary LA on tissue AA and failed to observe a positive relationship (111, 112). For example, by maintaining constant intake of ALA and increasing LA levels from 3.8% energy to 10.5% energy using LA-rich oils, LA was inversely associated with tissue AA levels ($p<0.001$) (47). These results were consistent with another study that reported an inverse relationship between dietary LA and tissue AA (112). This latter paper was not included in our review because it did not meet all of our inclusion/exclusion criteria, nevertheless it clearly showed that dietary LA does not increase tissue AA content. A number of studies included in this analysis also reported significant inverse relationships (103, 113, 114, 116). The data suggests that as LA increases in the diet, it maybe be competing with AA for reacylation into phospholipids.

A small number of studies modified LA intakes by using oils that also contained some ALA, such as soybean and canola oil (103, 117, 119), but the results from these studies were not significant and were similar to the other results. It must be remembered that soybean oil has a LA:ALA ratio similar (8:1) to that found in the US diet (10:1). We also included two studies that supplemented LA with recommended fish restrictions (because they met our inclusion/exclusion

criteria) (96, 118). One study (+176% LA) reported no changes in AA levels, while the other (+86% LA) reported a 10% increase in AA.

Some of the weaknesses of this review are reflected in the studies that qualified for our evaluation. Most were not designed to specifically address our research question; however, those that were specifically designed to evaluate the effect of dietary LA on tissue AA content yielded results that were similar to the overall results (111). Each study used a different population with potentially different background diets, but this would better reflect the consumption patterns of the general public. Not all studies were blinded (61% were blinded) and dietary LA was not exclusively modified. The methods for modifying LA intakes were varied and other dietary PUFA were not controlled for with the exceptions identified previously, and data for only two tissues were evaluated. When LA was modified, it was done so by typically changing the levels of an oil rich in LA (i.e., corn oil, safflower oil, sunflower oil) or foods containing LA (as opposed to adding pure LA), reflecting how the LA would be consumed by the general public. There was no standard length to the studies. For example, studies involving plasma/serum ranged between 14 days-5 months, and those looking at erythrocyte data ranged between 14-180 days. Importantly, the subjects were used as their own controls, the studies addressed changes in LA in relationship to Western-type diets, and the results were not different between those studies that were double-blind randomized placebo controlled trials (1/3) and those that were not. Despite these weaknesses, positive results were still identified with intakes of GLA and AA, helping to support those results reported with LA.

In summary, elevated tissue AA levels are believed to be positively associated with eicosanoid formation and risk for a variety of chronic diseases, including cardiovascular disease, cancer and inflammation. The literature expresses concern over the fact that increasing dietary

LA can potentially enrich tissues with AA due to their metabolic link. The results of this study do not support this concern. There is insufficient evidence to suggest that changes in dietary LA will modify tissue AA content in an adult population consuming a Western-type diet.

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Conflicts of interest: There are no conflicts of interest to report.

Contributions of the authors: BR conducted the research and co-wrote manuscript, and JW formulated and designed research, co-wrote manuscript and had final responsibility for all parts of the manuscript.

Chapter 3

3. Summary and Conclusions

AA is arguably the most important PUFA associated with membrane phospholipids. Upon release, it can be enzymatically converted to potent bioactive lipid molecules called eicosanoids. Eicosanoids are linked to a variety of chronic diseases such as CVD, cancer and inflammation (48). Furthermore, tissue AA abundance is directly correlated with eicosanoid production (14). What is less understood and more controversial is the impact of dietary precursors of AA (i.e. LA) on changes in tissue AA content. The literature expresses concern with regards to the high n-6 PUFA content in the Western diet, *viz.*, LA, and how high intakes might lead to further enrichment of AA in tissues and subsequent eicosanoid production (150). As such, to lower tissue AA levels, recommendations emphasize reducing the levels of LA in the diet. To address this concern, a review of the literature regarding this issue may provide insight as to whether tissue AA is modified by dietary precursors (i.e. LA).

Studies involving dietary interventions that modified LA intake were sought from the literature. The initial search produced 4336 abstracts. Using our inclusion/exclusion criteria, 293 articles were retained and further reviewed. Eventually, 36 articles met all necessary criteria for retention. Data on LA supplementation was analyzed as % change based on calories (increasing or decreasing from baseline) or the levels supplemented (g/d).

The results demonstrated that no significant correlations were found between changes in LA consumption and changes in tissue AA content when expressing the data based on % calories increasing or decreasing) or g/d supplementation. Two possible reasons could explain the results, *(i)* feedback inhibition on the conversion of LA to AA via the delta-6 desaturase step, and/or *(ii)* tissue saturation of AA. To address these possibilities, we investigated articles that supplemented GLA in the diet. GLA is an n-6 PUFA that is introduced after the delta-6 desaturase step. By supplementing diets with GLA, it resulted in AA enrichment in tissue phospholipids, suggesting

the ability of dietary LA to modify tissue AA was being controlled by the delta-6 desaturase step. When AA was supplemented to the diet, further enrichment occurred, suggesting subsequent secondary regulatory steps (i.e., delta-5 desaturase) are involved in AA formation from precursors.

In conclusion, these results indicate there is insufficient evidence to suggest that tissue AA content is affected by changes in LA intake in the context of a Western diet. This affect appears to be based on feedback inhibition of delta-6 desaturase. However, by-passing this regulatory step with dietary GLA results in further enrichment of AA, apparently regulated by delta-5 desaturase. And finally, of all the n-6 PUFA in the diet, tissues are most responsive to dietary AA.

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APPENDIX

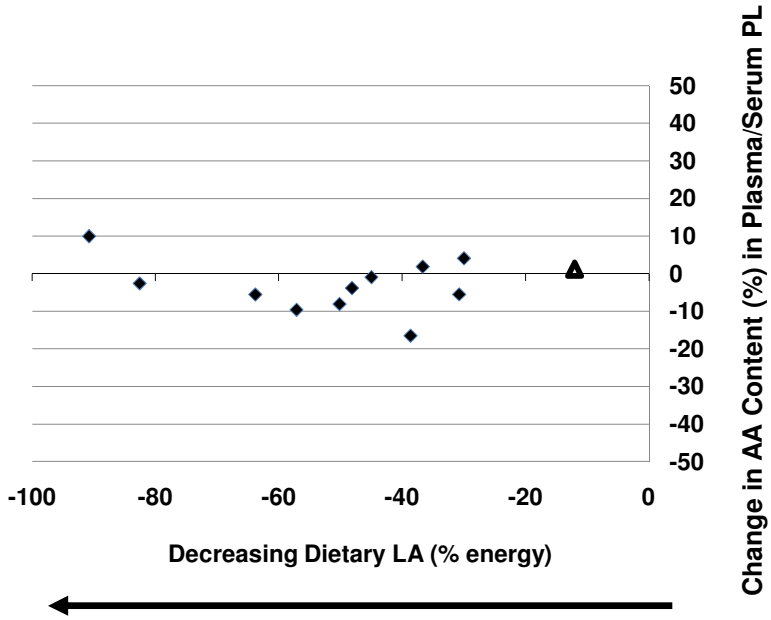

Figure 4: Effects of decreasing dietary linoleic acid (LA) intake (% change) based on energy on changes in plasma/serum phospholipid arachidonic acid (AA) content. Significant changes ($p<0.05$) in AA as reported in the original papers are designated as triangles. Non-significant AA changes as reported in the original papers are designated as diamonds. Abbreviations: AA, arachidonic acid; LA, linoleic acid; PL, phospholipid.

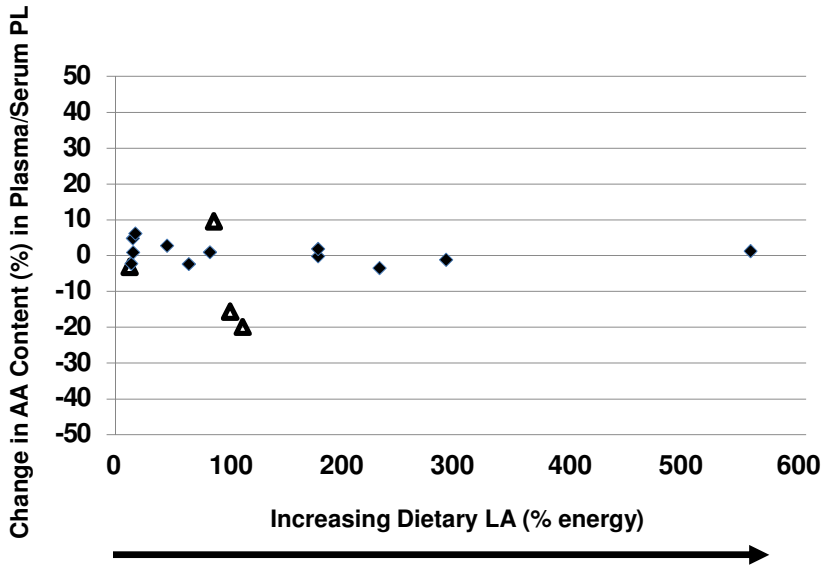

Figure 5**:** Effects of increasing dietary linoleic acid (LA) intake (% change) based on energy on changes in plasma/serum phospholipid arachidonic acid (AA) content. Significant changes ($p<0.05$) in AA as reported in the original papers are designated as triangles. Non-significant AA changes as reported in the original papers are designated as diamonds. Abbreviations: AA, arachidonic acid; LA, linoleic acid; PL, phospholipid.

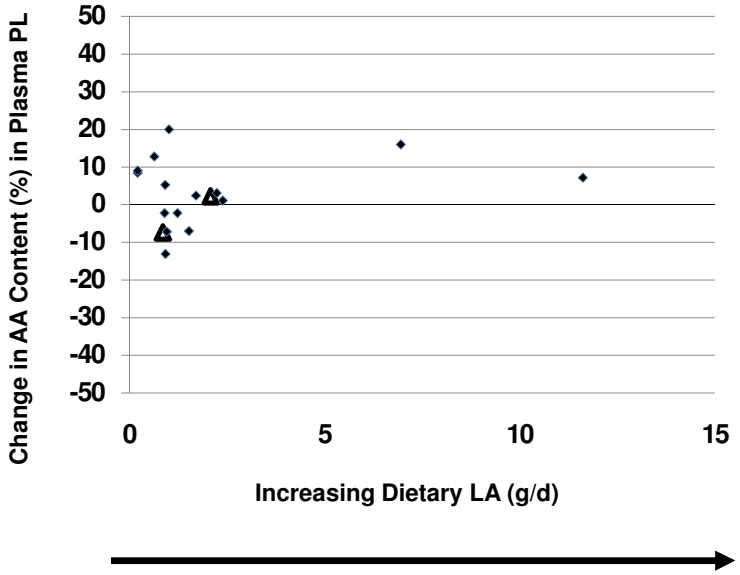

Figure 6: Effects of increasing dietary linoleic acid (LA) intake (g/d) on changes in plasma/serum phospholipid arachidonic acid (AA) content. Significant changes ($p<0.05$) in AA as reported in the original papers are designated as triangles. Non-significant AA changes as reported in the original papers are designated as diamonds. Abbreviations: AA, arachidonic acid; LA, linoleic acid; PL, phospholipid.

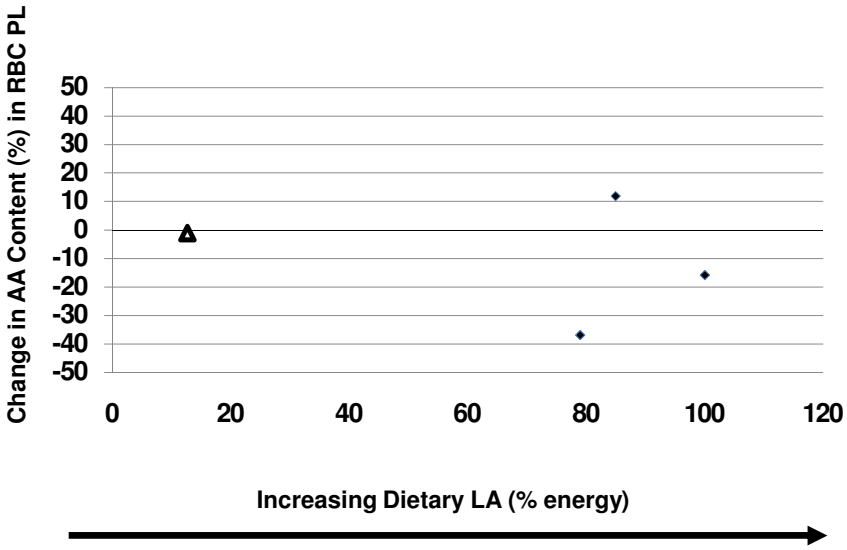

Figure 7: Effects of increasing dietary linoleic acid (LA) (% change) intake based on energy on changes in erythrocyte (RBC) phospholipid arachidonic acid (AA) content. Significant changes ($p<0.05$) in AA as reported in the original papers are designated as triangles. Non-significant AA changes as reported in the original papers are designated as diamonds. Abbreviations: AA, arachidonic acid; LA, linoleic acid; PL, phospholipid.

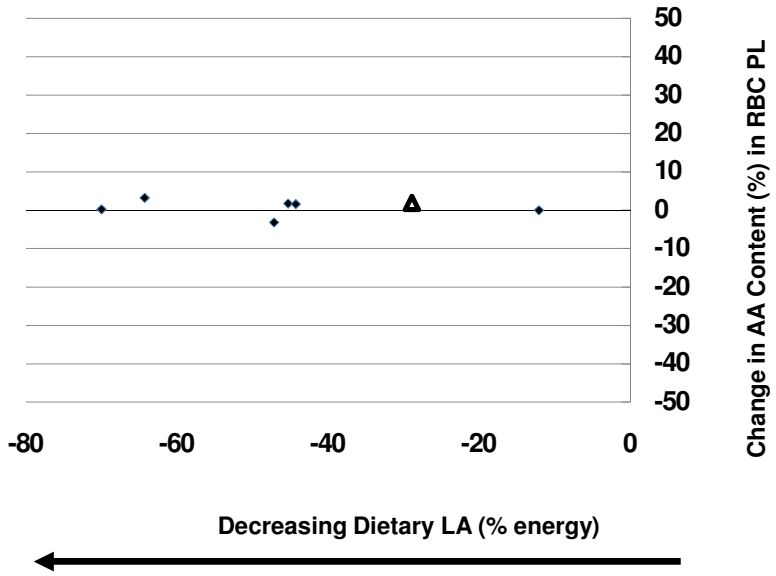

Figure 8: Effects of decreasing dietary linoleic acid (LA) (% change) based on energy on changes in erythrocyte (RBC) phospholipid arachidonic acid (AA) content. Significant changes ($p<0.05$) in AA as reported in the original papers are designated as triangles. Non-significant AA changes as reported in the original papers are designated as diamonds. Abbreviations: AA, arachidonic acid; LA, linoleic acid; PL, phospholipid.

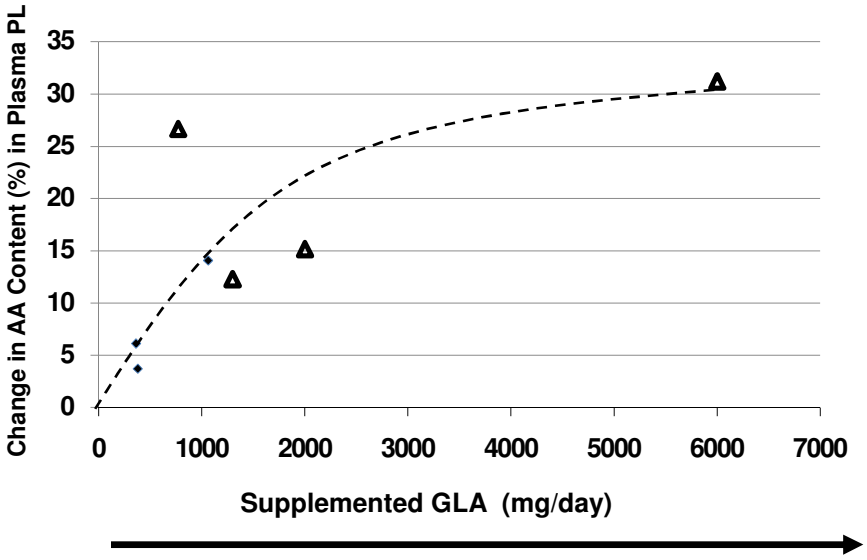

Figure 9: Effects of increasing dietary gamma-linolenic acid (GLA) (mg/d) on changes in plasma/serum phospholipid arachidonic acid (AA) content. Significant changes ($p<0.05$) in AA as reported in the original papers are designated as triangles. Non-significant AA changes as reported in the original papers are designated as diamonds. Abbreviations: AA, arachidonic acid; GLA, gamma-linolenic acid; PL, phospholipid

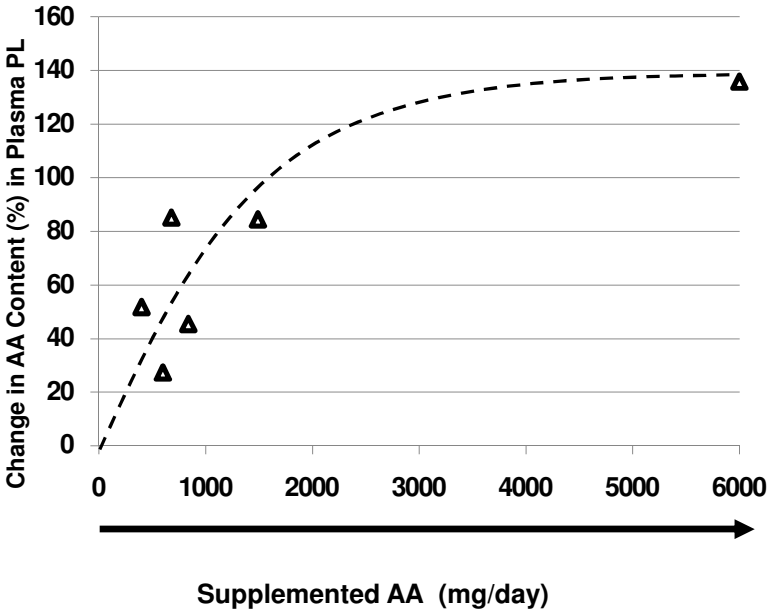

Figure 10: Effects of increasing dietary arachidonic acid (AA) (% change) based on energy on changes in plasma/serum phospholipid AA content. Significant changes ($p<0.05$) in AA as reported in the original papers are designated as triangles. Non-significant AA changes as reported in the original papers are designated as diamonds. Abbreviations: AA, arachidonic acid; PL, phospholipid.

VITA

Brian Rett was born in Florida and moved to Colorado before starting public school. He moved to Laramie, Wyoming in 2003 to begin his college education. He majored in Nutrition and graduated in 2008. In that same year, he was accepted and attended graduate school at the University of Tennessee. He has earned a Master of Science degree in Nutrition in May 2011. Future plans include a doctoral degree.